



3 April, 1975

Concentrate on fundamentals

"WE are here today", said Mr Ian Lloyd as he opened the Science Sub-Committee hearings on the needs of scientific research in British Universities, "to look into the funding of scientific research from all sources, public and private. The University Grants Committee, the Research Councils, charities and foundations—we shall investigate the distribution of funding amongst them. We shall also look at the relation of research to teaching, and the implication of changes in funding on research." To hear the six witnesses, there were three MPs, four pressmen and one member of the general public. Hardly an auspicious beginning to a penetrating survey.

On display were representatives from two of the newer and more technologically-oriented universities—City and Brunel. It was difficult to discern any very clear threads from their presentations. There was a certain amount of special pleading for things like tenured technicians, more travel money and less accounting duties, but the array of professors lost a major opportunity to put sharply in focus the plight of universities. Instead they indulged in a rambling dialogue in which important points were left unmentioned or given inadequate emphasis. Out of it all emerged a picture of two universities whose professors have slight chips on their shoulders about status and have a general desire to do something about the Science Research Council, but no very clear idea what they would do.

The most worrying impression left was that whilst the educational system faced crisis and some departments are denuded of students, there is no serious thought given to a strategy for universities or any well marked-out policy options which are being discussed in common-rooms. If universities themselves cannot present compelling evidence that they are thinking carefully about policy, they must expect that policy will be decided for them.

The primary need of scientific research is a continuous flow of highly intelligent manpower, and it is to this problem that the committee should pay greatest attention, for if the numbers coming into science at the undergraduate level continue to decline, then the *raison d'être* for many university staff disappears and any call for new facilities and equipment or even for the long-term financial commitments that every Vice-Chancellor dreams of landing is bound to be muted. Unfortunately, few in

the university environment have yet come up with much original thinking either on how to reverse the decline or what to do if the decline proves irreversible. There are, of course, various crumbs of comfort; up to the present the loss in recruitment has been mainly of the less qualified, and up to the present it has been possible to fill classes up with overseas students. But both of these situations could change within a year or two for the worse.

Although a City representative did touch on the "disastrous shortfall" in native-born applicants for graduate studentships, he did not pursue any line of thought on reasons why or remedies considered—nor, one suspects, will many of those who come to give evidence. It is unthinkable that a business which saw a steady decline in demand for its services would not go to great lengths to learn about the trend and try to respond to it; the committee would do well to ask some plain questions of future witnesses on their understanding of the decline in recruitment to science—maybe to the extent of asking whether the trend towards producing 44 equal universities (and even moving the polytechnics up to a level footing) is an intelligent response to a situation in which the science departments of half these universities are gasping for students.

Future witnesses might also be at some pains to spell out their views on the purpose of a university. Not unnaturally, in times like these the committee is pursuing rather doggedly the question of science in the national interest and the need for better industry/university links. Science for the country is a particularly tricky issue and needs some careful prior thought. If the laboratories really are full of overseas students, and if the international corporation, capable of exporting technology at will, increasingly dominates science-based industry, then talk of national interest is bound to become of diminishing relevance.

Certainly the international context of science—even the European dimension—is a difficult idea to get through to politicians who obviously want to see returns for taxpayers' money. And yet to fail to make the broader scene clear would be to run the risk that university science departments be forced to pay too much attention to presumed national needs—and too little to the advancement of learning. □



international news

CONTINUING its investigation of the way in which energy is being conserved in the UK, and at the same time reinforcing its disbelief at what it sees as a "surfeit of inconclusive thinking" on the part of the government, the Energy Resources Subcommittee of the Select Committee on Science and Technology last week turned to the chemical industry, represented by three members of the Chemical Industries Association (CIA) Limited.

The subcommittee was clearly anxious to hear firm advice from the CIA about what sort of measures the government should be taking to see that energy is not wasted. In the event, what it got were some general comments about price restraint and taxation, but few specific recommendations.

The CIA did say, however, that the chemical industry in the UK expects to reduce its energy requirements per unit of output by about 10% by 1980, principally by good energy 'housekeeping'—maintaining a watchful eye on the amount of lighting used, unnecessary leakages of steam, the quality of insulation and lagging, and so on. Capital investment also helps, but usually only in situations where the increased cost of energy has made it economically sensible to replace equipment before it is worn out. As the association pointed out, although energy costs to the chemical industry have gone up by about 80% between 1973 and 1975, capital costs are not far behind with an increase of some 55%.

The CIA emphasised, however, that improvements in chemical manufacturing techniques could make for extensive energy savings. Some 4 million MW h of the 19 million MW h of electricity

British energy users' views

by Roger Woodham and Eleanor Lawrence

used by the chemical industry in a year—equivalent to about 460 MW out of 2,200 MW on a continuous basis—goes in the manufacture of chlorine alone, the CIA stated by way of an example, and the efficiency of that process has been increased in recent years by some 5% as metal anodes have come to replace carbon ones. Quite a respectable saving of energy.

Mr G. G. Harrison, of ICI, also confirmed the need for courses in energy awareness for people entering and working in the chemical industry.

● Following the CIA, the Energy Subcommittee took evidence this week from another massive energy user, the British Steel Corporation (BSC). With an annual total fuel bill of £600 million and a 10% share of Britain's total energy requirements, the steel industry is obviously in a position to affect the government's energy-saving plans considerably.

The main burden of BSC's argument was that modernisation of its plant and processes is essential if any further saving in energy consumption is to be made. If modernisation could go ahead quickly, the BSC reckoned on reducing the energy costs per tonne of steel produced by 10% by 1978.

Unlike many industries where energy costs have previously been only a few percent of total basic costs, British Steel has always been aware of the

possibilities of a more efficient use of energy. Since 1967 it has made a reduction of 13% in its energy requirements, but the 10% reduction envisaged up to 1978 would represent a much stepped-up effort. Asked about the possible shortage of qualified fuel engineers, BSC's Director of Research and Development admitted ruefully that the energy crisis and the growing need for fuel engineers had meant a massive turnover in BSC's own engineering force as it was one of the few sources of trained, experienced fuel engineers in the country. He envisaged that a much more extensive in-house training scheme would be necessary in the future.

A long term possibility for minimising energy costs for the steel industry is to harness the heat-requiring reactions (basically the reduction of iron ore) directly to the process heat from nuclear reactors without the intermediate generation of electricity. Unfortunately for the steel industry in Britain, the only reactor capable of reaching the required temperatures of around 1,000 °C is the high temperature reactor, which is not exactly a high priority in Britain's nuclear research programme. The Select Committee appeared slightly pained to learn that we should have to import this type of reactor technology from the USA, Japan or Germany in about 15–20 years when the link-up has been sufficiently developed. But as BSC gently pointed out, it was hardly fair to expect a commercial reactor to be developed without a market for electricity generation. This market has now been cornered by the SGHWR, the Select Committee's own choice. □

In a further step forward in the British government's efforts to cut defence expenditure from 5.8% of GNP in 1974 to 4.5% in the late 1970s, the Statement on the Defence Estimates, 1975 (Cmd 5976, HMSO, £1.22) announces more explicitly the targets to be aimed at. Previous plans were for expenditure (at 1974 prices) to rise from about £4,000 million for 1975–76 to £4,300 million in 1978–79 and £4,450 million in the 1980s. Now the aim is to cut £300 million a year off the budget in the next few years and eventually, in the 1980s, to be paying only £3,790 million for defence.

Research and development expendi-

ture is also to be cut fairly heavily. Throughout the 1960s the proportion of the defence budget devoted to it declined steadily from 15.5% (1961) to 9% (1970). Since then there has been a steady rise back to 12% and the plans were for it to stay at that figure until 1978–79 and then decline to 10% by the mid-1980s. Revised plans call for no substantial change in these percentages, but in absolute terms £440 million (1974 prices) less will be spent in the coming decade on research and development. Research accounts for about one-sixth of the expenditure, and of that sixth about one-fifth is called 'general or basic' research. One-

third of expenditure on research and development goes to aircraft, another third to guided weapons and electronics; 38,000 civilians and 1,500 military personnel work in the research and development field.

Part of the cut in expenditure that will be called for should come from the rationalisation process now going ahead in research and development establishments. The plans are for four 'systems' establishments for the four environments sea, land, air and under-water, and a number of 'technology' establishments dealing with service-wide requirements such as communications or propulsion.

THE final chapter is yet to be written in that 50-year squabble between the Administration and Congress over whether or not the United States should ratify the Geneva Protocol, which prohibits first use in war of chemical weapons. Although the Senate voted to ratify the Protocol in December last year and President Ford signed it on January 22, legal doubts about the Administration's interpretation of its terms have delayed the final stage in ratification—the articles have not yet been deposited with the government of France, which means that the United States is still not a formal party to the treaty.

The problem, according to sources in the Administration, involves the question of whether or not herbicides and tear gases are covered by the Protocol. When President Nixon resubmitted the treaty to the Senate for its approval in 1970, he did so with the understanding that a formal reservation would be written into the ratification, stating that the United States government believes that herbicides and tear gases are not included within the terms of the protocol. The Senate Foreign Relations Committee refused to accept that understanding, however, and an impasse developed until late last year, when a compromise was worked out between the Ford Administration and Senator William Fulbright, the chairman of the Foreign Relations Committee. It enabled the treaty to be approved unanimously by the Senate.

The compromise, in short, allowed the Administration to retain its belief that herbicides and tear gases are not covered by the Protocol, but no formal reservation to that effect would be written into the ratification. Instead, President Ford announced that he would issue an executive order setting out a 'national policy' that the United States would never be the first to use those agents in war, except in four minor instances (such as clearing undergrowth around military bases and controlling rioting prisoners of war).

The executive order has, however, never been issued because it is under legal review in the Justice Department, and until that review is completed, the articles will not be deposited with the French government. The review was initiated by President Ford's legal counsel, Philip Areeda, because of doubts about the legal status of such a 'national policy'. Since the Justice Department has little experience in such international matters, the review is taking considerable time.

One Administration official last week described the affair as simply a "bureaucratic snafu" which would soon be resolved. He pointed out that the

Japanese government has adopted a similar interpretation of the Protocol, and its ratification of the treaty has not been challenged on legal grounds.

Nevertheless, it should be noted that the United Nations adopted a resolution in 1969 stating that the protocol covers use in war of all chemical agents.

● Meanwhile, the Department of Defense has, as expected, renewed its

Washington seen

by Colin Norman



request for Congress to provide funds to allow production of binary nerve gas weapons to begin. Last year, Congress refused to provide \$5.8 million for production of binaries, chiefly on the grounds that if the United States develops a new generation of nerve gas weapons, chemical disarmament talks now taking place in Geneva would be torpedoed. Undaunted, however, the Department of Defence is now asking for \$8.8 million for binary production.

It is considered unlikely that Congress will approve the request, because the arguments raised against binaries last year will be just as strong this year. Already, Mr Richard Ottinger, a liberal Democrat from New York, has introduced a resolution barring production of binaries and the campaign against the weapons is likely to be stepped up in the next few weeks.

● There are indications that the Food and Drug Administration (FDA) may partially lift its controversial ban on cyclamates in the next year or so. If so, the agency will bring down almost as much criticism as it encountered when it abruptly removed the sweetener from the market in 1969, following reports that it causes bladder cancer when fed in high doses to rats.

The latest development in the cyclamate saga occurred last month, when the FDA informed Abbott Laboratories, the manufacturer of cyclamates, that it would ask the National Cancer Institute to determine whether or not the sweetener is a carcinogen. The move represents a considerable retreat from the FDA's contention, announced in September last year, that insufficient

new data are available to justify reconsidering the ban.

According to a letter sent to Abbott by Richard J. Ronk, Director of the FDA's Division of Food and Color Additives, the question of whether or not cyclamates are carcinogenic "remains a difficult one to resolve". Although he noted that some FDA scientists believe that there is sufficient evidence to conclude that cyclamates do cause cancer in rats, Ronk suggested that "it is the apparent opinion of the oncological community of the world that cyclamates when tested in accordance with appropriate protocols are not carcinogenic".

On the day that the letter was sent to Abbott, EDA Administrator Alexander Schmidt asked the National Cancer Institute to set up a panel of cancer specialists "with impeccable credentials" to review the evidence on the carcinogenicity of cyclamates as soon as possible. The outcome of the cancer institute's review is crucial because a provision in the food and drug laws, known as the Delaney Amendment, forbids use of any food additive which is found to raise cancer in test animals. Unless the review comes up with the conclusion that cyclamates are not carcinogenic, there is therefore no chance that the sweetener will be brought back on the market.

But even if the National Cancer Institute gives cyclamates a clean bill of health as far as carcinogenicity is concerned, there are other doubts about the safety of the sweetener. The FDA's letter to Abbott notes, for example, that tests have shown that cyclohexylamine—a metabolic product of cyclamates—causes softening of the testes when fed to rats. Because of that, the letter hints that if the sweetener is allowed back on the market, the FDA would probably propose regulations to regulate intake to about 0.5 g per day.

Although the tone of the FDA's letter seems to indicate that the agency believes that cyclamates are not carcinogenic, a final decision is not likely to be made for at least a year. If the agency does lift its ban on cyclamates, a flood of complaints can be anticipated from Congress and from consumer groups, and the FDA is therefore trying to tread cautiously.

● Congress has, as expected, rejected President Ford's suggestion that \$351 million be cut from this year's budget for the National Institutes of Health. Both the House and the Senate have passed bills directing Ford to spend all the money appropriated for this fiscal year—which now has only three months left to run—and so, barring last ditch delaying tactics by the Administration, the money will now be made available.

Little progress for Indian education

from Narender K. Sehgal, Jullundur

EDUCATION is a subject that has nearly always been topical in independent India—probably because, in spite of all the discussion and debate over it, so very little has been done during the past quarter of a century. At present two-thirds of all Indians are illiterate. Yet large numbers of the educated, and the highly educated are unemployed, not to mention the multitudes of the uneducated unemployed and those who are underemployed. Such anomalies abound: there is an acute shortage of doctors in rural India but at the same time not only are thousands of Indian doctors working abroad, there is also widespread unemployment among medical graduates in the country; India badly needs middle-level skilled professionals and other types of trained worker suitable for rural areas—the kind our system is just not turning out. It appears that the Indian nation spends countless crores in educating and training individuals who only seem of value in foreign lands.

These facts speak volumes about education in India, among other things. The system of education—a legacy from the bygone colonial era—has been a constant target for criticism. And understandably so, because the present system differs little in content and character from the one the English rulers had devised with a set of aims and objectives of their own—quite different, one would think, from those which might have most suited a free nation.

In view of the general situation at the time of independence, the leadership thought it fit to maintain the *status quo* in education, as in many other areas, and to let the drastic changes wait till normality had returned. But this turned out to be a blunder, because as time went by those who took charge of education, perhaps incapacitated by their own background and training, did little or nothing to devise a new system or to modify and improve the existing one. As the Prime Minister, Shrimati Indira Gandhi, admitted as late as last year: "One of the biggest mistakes we made when we gained independence was not to have overhauled thoroughly our educational system and structure. We are paying for it now".

All that the government has been able to do in this direction till now is to appoint committees and commissions to go into various aspects of the system and structure of education. These bodies have produced tonnes and tonnes of words in the form of voluminous reports which are gathering

dust on shelves in the offices of bureaucrats in the Ministry of Education and elsewhere. One would like to believe that, armed with all these investigative and in-depth reports from eminent and learned men, the government would by now be quite clear about what needs to be done and how. This, however, is not the case. Confusion and uncertainty mark statements made from time to time by those in authority. The education policy of the government of India seems to be unclear and lacking in definite direction.

The Prime Minister herself has not helped matters by sounding inconsistent in her numerous speeches on the subject, and sometimes at variance with the approach educational planners in her own government would like to adopt. On more than one occasion she has explicitly agreed with the widely held view that the present system of education must be drastically changed, or even discarded altogether, as in her own words 'it has little contact with life in the country . . . It belongs not only to another civilisation, it belongs to another century. And we need a minor or even perhaps a major revolution . . .' On another occasion, not too long ago, she chose to caution educational planners against policies and drastic measures which would cause major 'dislocations' in the existing educational process. As recently as February 1, while speaking at the Silver Jubilee celebrations of the National Chemical Laboratory in Poona, the Prime Minister said: "With all the known shortcomings of our educational system and of our organisation of science, the fact remains that India pro-

duces exceptionally gifted scientists and an impressive number of highly competent scholars."

To cope with mounting unemployment among the educated youth, planners in the Central Advisory Board of Education (CABE) would like to adopt a strategy which stresses the vocational aspects of the educational process. This, besides enhancing chances of employment and self-employment through inducements like financial and technical assistance from various governmental and other agencies, will also help ease pressure on enrolment in institutions of higher learning—in line with another CABE proposal which seeks to place drastic restrictions on expansion in higher education. Again, Shrimati Gandhi in her Poona speech last month observed: "Many student groups come to see me and often argue that our educational system should be given a vocational orientation . . . But training a person for a job is not the sole or the highest purpose of education, nor is it adequate for the longer run. That purpose is to train better human beings who can give more to life and be capable of deriving more from life."

In regard to curbs on expansion in college and university enrolments (which have increased by 800% in the past 20 years), the Prime Minister cautions that it must not in any way result in limiting heightened expectations of those in the backward classes, and that such curbs on expansion of higher education must also be accompanied by concrete steps to bring about a change in the composition of the student population in such a way that weaker sections of society can get their fair share of opportunities.

Views expressed by the Prime Minister on a subject are generally looked upon as being indicative of the official line of thinking, if not as statements of official policy. So if Shrimati Gandhi's views are looked at in that light, we can look forward only to more of what has been going on for so long—more debate, more committees, more reports and substantive action, if any, so snail-paced that the passage of time will more than nullify its effects.

The Prime Minister's warning on 'dislocations' in the existing process of education was unfortunate and really unnecessary. For what use was it asking those who hardly move at all to go slow? And again, her linking of the present 'system' to the fact that India produces "exceptionally gifted" individuals was amusing because many Indians are able to achieve what they do in spite of the 'system' and not because of it. It may be more relevant and instructive to investigate the number of talented Indians this system helps send into oblivion or nip in the bud each year. □

SHEIK Zayed Bin Sultan Nahayam, ruler of Abu Dhabi, has a good line in greenhouses. They are soon to cover a 15-acre site in the desert in an attempt to start the first commercial market garden in the Gulf States. The greenhouses will be the plastic-dome type and the irrigation supply will be from water reservoirs that have been discovered under the desert by scientists from the French national oil company, CSP.

The great problem with desert irrigation is, of course, evaporation. This will be overcome by a recently discovered system in which hose pipes, punctured with small holes of calculated size and spacing, are laid out along the rows of plants. Water is thereby delivered at the optimum site and rate to minimise waste.

And who discovered the irrigation system? According to Israeli scientists it was devised at the Weizmann Institute (where it can be seen on the campus flower beds). Will Sheik Zayed give credit where it's due?

VERY recently, the first cigarettes at least partly made of substances other than tobacco were launched onto the market—in Germany and Switzerland. One brand contains an American substitute; the other a German-made one. Imperial Tobacco, which has 65% of the cigarette market in Britain and the largest volume turnover of cigarettes of any company in the EEC, now “hopes that its cigarette, containing its New Smoking Material (NSM) will be on sale to the public within 12 months. Imperial (in conjunction with ICI) has spent £6 million on developing this tobacco substitute and a £13-million factory to produce 30 million pounds of it a year is nearly ready at Ardeer, Scotland. The project is held up by the deliberations of the Hunter Committee—a body set up in 1973 under Dr R. B. Hunter to advise the Secretary of State for Health on guidelines for assessing and testing the risk/benefit ratio of the tobacco substitute; it has yet to report, though it gave Imperial the go-ahead for consumer acceptability trials of its product last August.

Presumably on the basis of these trials the expected “new-smoke” (to use an Orwellian term for an Orwellian concept) is not likely to contain more than 20% of NSM—the rest will be standard cigarette tobacco. I have smoked a cigarette made entirely from NSM and quite see the point. It is

supremely non-addictive. In fact it resembles nothing so much as lighting the wrong end of a filter-tipped cigarette—which is perhaps not too hard to understand since filters are made of cellulose and so is NSM with the difference that NSM is toasted to reduce the

Nosmoke without fire

from Angela Croome

water content and give it a nice dark tobacco colour. The brands launched elsewhere in Europe are much the same—it seems there is no substitute for wood—and one of the German lines has the appealing name “Nosmoke”.

But with 80% tobacco, “no-smoke” it isn’t. Nor, one hazards, would a cigarette without nicotine satisfy the complex needs that a cigarette smoker seeks. Curiously the massive research that Imperial has put into improving the smoking (including medical) characteristics of their cigarettes does not at present include measuring the nicotine level. Tar and nicotine delivery are lumped together, and come out at 28.30 micrograms per cigarette as against only 7 micrograms for a cigarette consisting solely of NSM. But Imperial’s

philosophy is explicitly that it is the tar that counts, and refers to the classical mouse-skin painting tests. The company claims that not only is there less tar from NSM but that it is a different tar. It is something that toxicology tests on NSM are being run by the independent laboratory with the greatest experience in toxicological testing, the Huntingdon Research Centre, and that screening there includes possible teratogenic effects. It would be even more comforting to know that the Hunter Committee was going to recommend measures and standards of carbon monoxide delivery, implicated in heart disease which smoking is considered to accelerate—and that the committee was going to publish its report which it has no obligation to do.

We are assured that NSM or “Nosmoke” or any other semi-substitute cigarette will not be cheaper—“the Government cannot afford it”; nor of course can the cigarette companies. So the new-smokes must be sold on the “better for you” argument which on the evidence so far is specious. Also one understands that cellulose from wood pulp (the nearest to a specific description of the new smoking material one can get) is cheaper than tobacco. There is no prize for guessing who is going to be better off if the cigarette-smoking public can be persuaded to switch.

It’s very exceptional indeed in the Netherlands that an essentially scientific dispute ends with people dragging each other into the courts. Yet this is what happened when two Groningen archaeologists, Drs H. Tj. Waterbolk and D. Stapert, accused a highly esteemed amateur, Tjerk Vermaning, of showing and selling fake Stone Age artefacts.

Vermaning, a 46-year-old mowing machine repairer who has been living on an archaeology grant for several years, replied that one does not readily imitate (for example) a few hundred stone axes, that his accusers are of no scientific standing anyway, that he wants some “real archaeologists” to look into the question, and that he will sue Stapert and Waterbolk for libel.

After being under arrest for two days, Vermaning was released.

The whole affair seems to be the dead-end of a continuing story of trouble-laden cooperation of amateur and scientist. Some ten years ago Vermaning gained national renown and became a local hero by digging up the remains of a Neanderthal camp site with several hundred artefacts in it, among which were various types of fist axes and a number of splinters. The Groningen archaeologists could even restore some of the original firestone knolls, from which Neanderthal man

Neanderthal axes to grind

from Arie de Kool, Rotterdam

had chipped his tools.

Vermaning was very proud of his finds and expected rewards—not just the few hundred pounds that were paid for the stones, but an honorary doctorate and a staff appointment at the institute. The university did not grant this to the astonishingly little educated man (three winters of elementary school, in summertime he had to help his parents), but they thought his talents sufficiently important to give him financial support. Altogether this amounted to some £20,000 over several years.

Vermaning decided, however, that this was not the recognition he was entitled to and he refused to co-operate further—after he had found a new site, again containing several hundred pieces.

Suspensions began to grow, said Dr Stapert, when on a site where Vermaning had found more than 400 pieces, the archaeologists were not able to uncover one single chip more. Vermaning uses this as an argument that the professional people are just not as com-

petent as he, but Stapert began to think that the 400-odd pieces that Vermaning found were just those he might have buried a short while before.

His suspicion was greatly enhanced when he found what he thought to be traces of machining on two of the artefacts, and he became certain when he discovered that the typical shiny surfaces, supposed to be caused by weathering, were what he described as an easily removable recent kind of lacquering.

He called in the police, accusing Vermaning of fraud—not just scientific but also financial, since he had sold recent imitations as genuine, old artefacts. Vermaning was arrested and scientists, police and provincial government called a press conference.

But Vermaning asked the police what proof there was in the statements of people who had refused to recognise him for his discoveries, which amounted to many times what they themselves had been able to dig up. At the same time he accused the scientists of libel and demanded impartial evidence.

In the Netherlands people are wondering who did make the axes, if not Neanderthal man, and also whether it was wise to cause a public rumpus before the matter had been dealt with in court. □

UNDETERRED by the Arab boycott, an American firm with a stake in local science-based industry this week announced that it will be increasing its activities in Israel. The firm, Miles Laboratories of Elkhart, Indiana, plans to invest an additional £8 million in its Israeli subsidiaries, which produce everything from the company's famous Alka-Seltzer to a broad range of sophisticated chemicals for research laboratories.

Two of the Miles' ventures in Israel are closely linked with local institutions of higher learning. Miles-Yeda, located next to the Weizmann Institute in Rehovot, makes research chemicals based on Institute know-how. These include lectins, spingolipids, affinity chromatography material, peptides, O^{17} and O^{18} stable isotopic compounds and a considerable number of immunochemicals. At Ames-Yissum in Jerusalem, where Hebrew University research is utilised, diagnostic kits for testing the thyroid level in blood are the main product. Export sales of these science-based firms last year hit £1.2 million.

Miles, a "non-Jewish" company, was not attracted to the country by sentiment but by the chance of making a profit. The Israeli Government realises this and does its part to encourage science-based industries by giving them loans and grants, as well as by fostering the establishment of special science-based industrial parks near universities and research centres.

The conditions offered are apparently so attractive that many enterprises which could hardly be called science-based try to push their way into these parks. In order to ensure that the parks serve the purpose for which they were established, the Ministry of Commerce and Industry announced this week that only enterprises which have ties with nearby academic centres would be allowed into them. Those firms already in the parks will only be allowed to expand if they carry on a substantial R & D programme of their own.

As elsewhere, orders from the military have played a key role in the development of science-based industry, notably in the electronics field. Some achievements in electronics have been publicised; others are still under wraps.

Publicity often comes when the firms concerned seek to enter the export market. This is probably the reason why Israel Aircraft Industries, which already has multimillion pound overseas sales of rockets and planes to its credit, last week unveiled the Autocommander, a supersonic automatic pilot.

Fully automated, the Autocommander relieves pilots of mechanical

flight routine and enables them to concentrate on target pinpointing and other tasks while flying at more than twice the speed of sound. It incorporates a reliable and fast-working detection system, including dual circuits, which ensure smooth functioning even if part of the device should fail. Now being produced for the Israel Air Force, the Autocommander may become a major export item.

● The military situation has also given impetus to research on war-related medical problems, such as that conducted by Professor Arye Weinrab of the Hebrew University and Mr Ezra Loewinger of the Hadassah-Hebrew University Medical Centre on the detection and identification of metal

Letter from Israel

from Nechemia Meyers

splinters which enter deep into the eyeball as a result of accidents or battle injuries. Exact information about such splinters is important for surgeons, who must decide whether a delicate and difficult operation on the vitreous body of the eye must be performed.

If the metal in the eye is steel, it may be removed by an electromagnet. Chips of many other metals, though a source of irritation, can be left in the eye as they do not cause biochemical damage. Copper splinters, however, present a special problem. Sometimes the fragment of copper becomes encapsulated in the vitreous body of the eye and is rendered at least temporarily harmless. On other occasions, the fragment of copper dissolves, releasing copper ions which, if they reach a certain level, can cause metal poisoning and an eventual loss of sight. The doctors therefore require some means of keeping a constant watch on the copper level.

Weinrab and Loewinger developed a system to detect the presence of metal ions in the eye by means of X-ray fluorescence. The radiation is measured by a solid state detector which translates the X-ray energy into electric pulses of varying intensity according to the metal present. Before the Yom Kippur War they were doing experiments on rabbits; when the war broke out and soldiers with eye injuries began pouring into Israeli hospitals, the metal detecting test was tried out on them.

In many of the cases involving tank crew members, copper was found. It came not from the tank itself (which is made of steel), but from the copper sheath near the head of the Russian anti-tank missiles hurled at the tanks. The copper melted at the high temperature of detonation, so almost every steel splinter in a tankman's eye was

either encased in copper or had copper adhering to it.

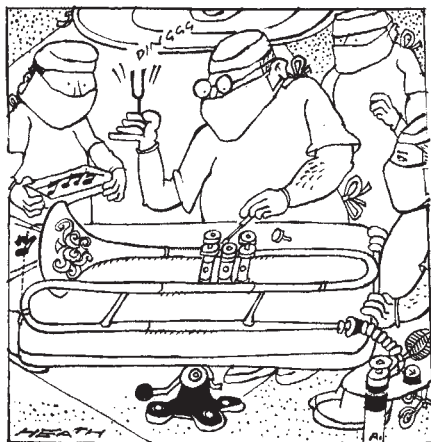
● The severity of eye injuries during the war has led some of the country's leading ophthalmological experts to recommend that all members of armoured and motorised units wear a special plastic shield. Such a shield, already used by Israel Air Force pilots, is made of a plastic compound ten times more resistant than glass to high velocity particles. It is also light, translucent and does not cloud with water vapour.

● This shield will, of course, be of no benefit to men who have already lost their sight in combat, but attention is also being paid to their problems. Chemist Avraham Schwartz of the Defence Ministry's Weapons Development Authority, using spinoff from research on plastic materials, has developed a process for printing books for the blind cheaply, quickly and in multiple copies. In the new process, typewriters punch tapes similar to those of teleprinters, instead of pressing out the usual Braille reliefs. These tapes are mounted on paper, and plastic is poured over them, penetrating the holes. The raised dots that result can be printed on both sides of the sheet of paper, which itself serves as a relief plate for reproduction of the "printed" material.

● The recently suspended talks between Israel and Egypt prompted several dozen scientists to recall an off-the-record discussion they had had some ten years ago with Henry Kissinger, then visiting Israel as a private citizen. It was a discussion with special relevance to the question of how much trust can be placed in US guarantees of an eventual settlement, should one be reached.

The conversation occurred at a time when Kissinger was campaigning against the proliferation of nuclear weapons, and so he naturally urged the scientists present to oppose the development of such weapons by Israel. When Kissinger's presentation was completed, one of the Israelis put forward a question present in everyone's mind. "If," the scientist said, "we were to accept your advice and forego the development of atomic arms in return for an American promise to assist us should we be under threat of attack by a nuclear power, could we be sure that such a promise would be kept?"

After pondering the query for a few moments, the Harvard scholar answered: "No, you couldn't be sure. American policy is made on an empirical, day-to-day basis. It would depend on the circumstances of the situation when the crisis developed." One wonders what kind of an answer Kissinger would give today.



Note paper follows?

A member of the physics department at the University of Surrey, Dr John Bowsher, must be working on one of the most unusual projects to be sponsored by the Science Research Council. He is to bring the scientific method to bear on the burning problem of why really good trombones sound the way they do. All trombones, it would seem, do not sound the same even though they may look indistinguishable, and it is generally the skill of a craftsman who knows just where to change the shape of a given instrument slightly

that saves many new instruments from the ignominy of being $\beta+$ rather than α .

With the music company Boosey and Hawkes, who produce 300 trombones a week, relying on the skill of just one craftsman in his 50s to suggest a little judicious adjustment in such and such a place, Dr Bowsher seems almost to be working against the clock in his analysis of the 'transient qualities' of trombone notes—namely what goes on in the first and last few milliseconds. Fortunately Dr Bowsher and one of his research students are actually trombone players themselves. □

correspondence

Czech conditions

SIR,—The discussion in *Nature* on the plight of the Czechoslovak scientists and my participation in it has had very unexpected and grave consequence for me personally.

A few days ago I learnt of a decision of the Czech Ministry of Inner Affairs, according to which I have been stripped of my Czech citizenship. The motivations for such an extraordinary decision are given as follows:

(1) The lecture about human rights I gave at the annual meeting of the German section of Amnesty International in June 1974 in Duisburg. (2) My letter to *Nature* (September 20, 1974), which, I have now learnt, had been broadcast in Czech by Rado Free Europe.

Needless to say, I protest strongly against this decision, which I consider unlawful and contradicting the Universal Declaration of Human Rights as well as other generally valid and accepted declaration and principles.

After being unemployed for more than three years because of my public activity and statements I was allowed to leave Czechoslovakia temporarily in December 1973. When leaving my country I submitted letters to the President of the Czechoslovak Academy of Sciences and to the Ministry of Education, informing them that I was leaving only because I could not find a proper job there, that I would be working abroad as a Czechoslovak scientist and citizen, and that I wished to return home as soon as a job was offered to me.

The decision of the Prague authorities provides another strong argument against the opinion that things are being normalized in Czechoslovakia; it shows what is the real situation and the con-

ditions Czechoslovak intellectuals have to live in.

Yours faithfully,

FRANTISEK JANOUCH

Stockholm

Citation analysis

SIR,—Concluding their recent letter (March 13), Pragier and Ronayne, citing Langrish, state "... our results raise serious doubts as to the validity of citation analysis in the determination of the relationship between science and technology."

I suggest that these conclusions have been reached because of a misconception about the scope of citation analysis, and do not, in fact, cast doubts on validity.

Langrish examined citations from articles by British industrial chemists, finding that they were largely of non-university origin, whereas Pragier and Ronayne studied citations from biologically oriented articles in *Reports on the progress of applied chemistry*, finding them to be mainly of university origin.

From this it may be concluded, as Pragier and Ronayne state, that information was drawn from different sources by these two communities, but this does not mean that such studies are not of value for studying the background of university and industrial science and technology.

What it does mean is that such studies are likely to be most useful when applied to a community whose members are characterised by having an interest in a specific aspect of science.

If the same pairs, triples, quads . . . , of references are observed in a number of articles, there is an implied consensus of opinion—the authors are perceiving a relationship between the earlier cited

articles. The heavily co-cited articles form the putative 'core' literature of the subject. This technique, originated by H. Small, was used to study the literature generated by the scientific community engaged in amorphous (chalcogenide glass) semi-conductor research from 1968 to 1973. Until 1972 the core articles, with one exception, came from universities.

In 1972 the core was augmented by articles from non-university sources. The cause of this was heavy co-citing of 1972 articles by 1973 authors. These authors often used title words indicating that device applications were described in their articles. It may be concluded that during this period materials, hitherto of research interest, started to



A hundred years ago

Dingler's Polytech. Journal contains an account of researches made by Dr Otto Krause, of Annaberg, on tobacco smoke, which he finds contains constantly a considerable quantity of carbonic oxide. The after effects of smoking are said to be principally caused by this poisonous gas, as the smoker never can prevent a part of the smoke from descending to the lungs, and thus the poisoning is unavoidable. The author is of opinion that the after effects are all the more energetic, the more inexperienced the smoker is, and he thus explains the unpleasant results of the first attempts at smoking, which are generally ascribed to nicotine alone. from *Nature*, 11, 456; April 8, 1875

be used in devices having practical applications.

Articles identified in this way may be depicted as interconnected elements on a citation map. Replotting of the maps periodically enables the growth, decay and changing interrelationships of specialities to be studied. It seems likely that this will provide an overview of the ramifications of science for the benefit of science policy makers and others.

A. E. CAWKELL

*Institute for Scientific Information,
Uxbridge, UK*

Great Plains weather

Sir,—A number of authors have pointed out the periodicity or quasi-periodicity of 20 to 22 years in the recurrence of droughts in the Great Plains of North America, the region between the Rocky Mountains and the Mississippi River. Perhaps most notable of these droughts was the "Dust Bowl" era in the 1930s which retarded the Great Plains agriculture and economy for a full decade. Most authors have also pointed out that the mid-years of the droughts coincide fairly well with every other sunspot minimum, specifically the minimum in which the polarity of the leading spots in the Sun's northern hemisphere is changing from north-seeking to south-seeking.

Since we are now approaching another solar minimum of the type mentioned, the question of the reality of the sunspot drought relationship is of great interest. The dates of the four most recent solar minima of this kind, and the central year of the four most widespread droughts in the Great Plains, were as follows:

Sunspot minima	Mid-years of droughts
1889	1892
1912	1912
1933	1934
1954	1953

The causes of these droughts are not well understood. J. R. Borchert has pointed out that some of these in the table were associated with greater than normal zonal circulation of the atmosphere, and some with increased meridional flow, evaluated on a hemispheric basis. The common element in drought situations seems to be stability in the type of weather pattern, that is, drought tends to be associated with a highly persistent pattern, either meridional or zonal.

I. R. Tannehill points out that drought in the Great Plains is associated with higher than normal pressure in the eastern Pacific area, whereas others find that droughts in western Kansas tend to occur simultaneously with positive height anomalies at 700 millibars over the eastern Pacific between latitudes 30° and 40° N and with negative surface temperature anomalies

over the eastern tropical Pacific.

In the spring and summer of 1974 there was evidence for the beginning of another drought in the Great Plains. If the situation is the same in most of the next several years, we may well conclude that the periodicity of 20 to 22 years is recurring. A sunspot minimum of the type mentioned; is expected in, perhaps, 1976.

In the spring the drought was confined to the western and southern parts of the Great Plains (Fig. 1). Most of the Great Plains region was actually suffering from too much rainfall, with serious delays to the starting of the spring planting in large areas. Meteorologically, the region was affected by greater than normal westerly wind flow. Thus the drought in the area just east of the Rockies may perhaps best be described as primarily a rain-shadow effect.

By mid-June and extending through July (Fig. 2), the situation had changed to a more meridional flow, with a stable high pressure cell over the Mississippi Valley in the upper atmosphere. This created an extended period of low precipitation and abnormally high surface temperatures just at the time when

certain crops, such as corn and soy beans, were reaching the maturing stage. For instance, Grand Island, in central Nebraska, reported no precipitation from June 15 until July 22, the longest rain-free period ever recorded at that station. The resulting damage to crops in such states as Iowa, Kansas, Nebraska, Oklahoma and adjoining states approached \$10,000 million dollars. This loss was caused mainly to the extreme but short summer drought, but also in part by the spring drought in western Oklahoma and northern Texas.

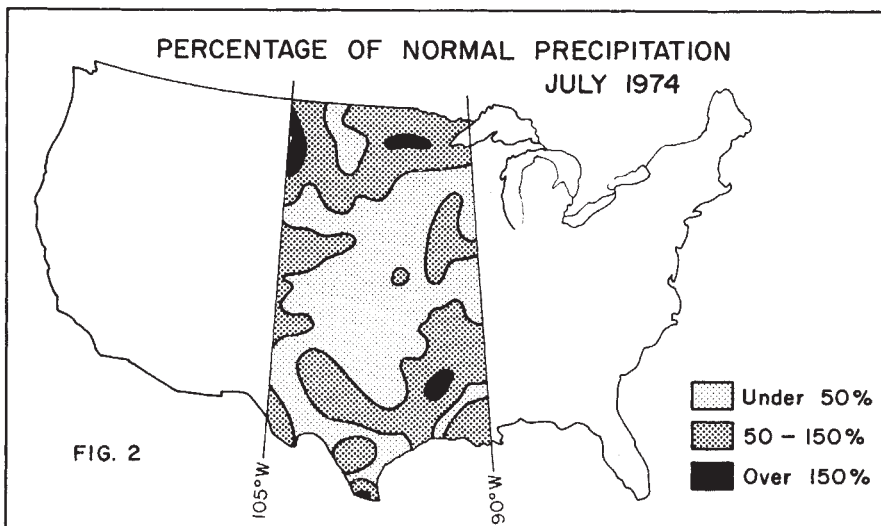
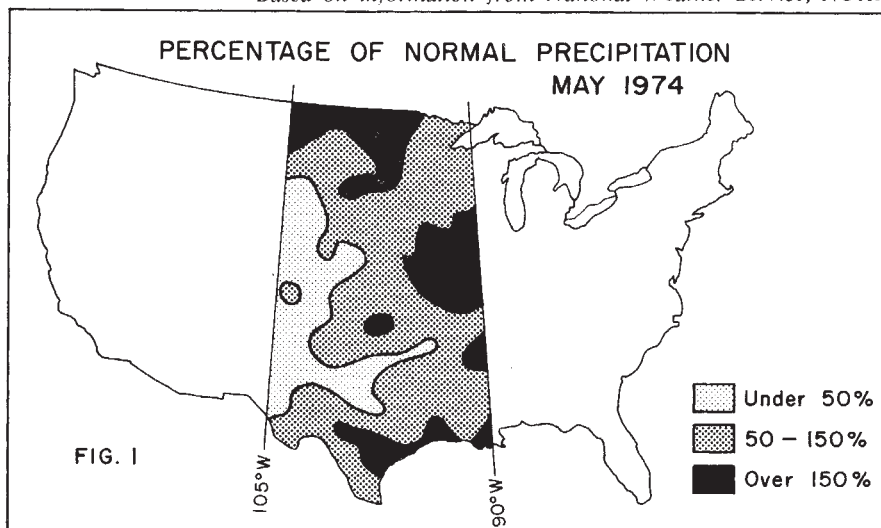
A mere coincidence in timing between the droughts and the double sunspot cycle will not, of course, constitute proof of a physical relationship. If, however, the drought of the 1970s does materialise, over the next several years, we will have a very strong incentive to search for physical mechanisms to explain the linkage or to provide other reasons for the recurrence.

WALTER ORR ROBERTS

ROGER H. OLSON

*University Corporation for
Atmospheric Research,
Boulder, Colorado 80303*

Based on information from National Weather Service, NOAA



news and views

Wider implications of catastrophe theory

from Henry Chilver

THE interesting article you publish today by Thompson on 'experiments in catastrophe' (page 392) prompts me to make a plea for wider study, by both physical and social scientists, of a very important area of 'systems analysis' in which the concepts of catastrophe theory may well have an important part to play. It will be evident to anyone who has attempted to 'design' a system—and particularly a complex system—whether this is in the field of engineering, organisation, planning, or in other fields, that modern design has encouraged designers to converge, in their ideas, on optimal systems.

The forces at work to encourage designers to converge on optimal solutions are very strong. All systems are based on ideas of increasing efficiency in one form or another: for example, in his article Thompson demonstrates how the search for the lightest (and therefore most efficient) structural forms leads to potentially catastrophic engineering structures. There are other examples in the fields of solids and fluids: the breaking of bonds between solid particles leading to the catastrophic failure of materials; the breakdown of smooth flow around bodies, leading to turbulent flow.

But there are many other types of systems—of widely different sorts—where catastrophe becomes more critical as design becomes more 'optimal'. Many transport systems are highly sophisticated and optimised—and therefore highly sensitive to small perturbations which may lead to collapse or catastrophe. One of the most sophisticated transport vehicles—the aeroplane—is itself a highly optimised system: its structure is highly efficient and its power in flight is highly sensitive to the reliability of its engines; more optimisation comes into the picture during the operations of the aircraft, and the history of the aeroplane is very sensitive to any perturbations which lead to loss of control and thus to catastrophe; again, this particular mode of transport is highly sensitive to weather conditions, and fog at the airport may mean diversion to other airports, and, thus, to disruption of the system.

But social systems themselves are 'planned' and 'designed' for equally critical states: one of our present social

problems is that we are optimising so strongly the complex interlocking and interdependence of institutions of many sorts that small perturbations of these systems can be catastrophic and lead to complete disruption. States of employment (and unemployment) of vital minority groups are clearly very sensitive to small changes of economic parameters, and this suggests we have built highly critical systems of social institutions.

Again, in manufacturing industry, the economy of production processes is highly sensitive to the volume of production, to the supply of crucial materials and components, and to the attitudes of small groups of staff. For this reason, the present world decline in trade is giving some industrial manufacturing companies serious problems, because their continuing good health is so sensitive to the scale of production. In such situations, sensitivity to small changes of the important parameters is wholly undesirable, but how can this be avoided if we are constantly forcing production processes to the extremes of low-cost and efficiency?

As pointed out by Thompson, Zeeman has shown some interesting catastrophe phenomena in natural systems. Alternative animal states may be compared with the bifurcating paths of classical stability theory, although in such cases it may be very difficult to give numerate values to the many 'axes' or 'vectors' involved. The state of good health of an individual is obviously an optimised condition—the 'design' in this case being reached through evolution and natural selection. In the course of evolution, many inadequate optimal states have been weeded out; a possible reason for their being weeded out is that they showed high sensitivity to small perturbations of their systems. In spite of this evolutionary process, the state of good health, in some respects, is still highly sensitive to a limited number of parameters, as, for example, to blood temperature and the working of a limited number of vital organs.

Some aspects of ecology may well present problems of potential catastrophe. The ecological balance in some systems is a delicate one: perhaps between tropical forest and desert, or between alternative forms of animal life.

In extending the concepts of Thompson, Zeeman and Thompson to a wider range of systems one is tempted to look for a common thread between all these problems. Following classical stability theory, we may search for 'potential functions' for all such problems, but this is not possible, since many of the common problems do not possess a simple potential function, nor, indeed, can be expected to possess them. Another approach would be to construct simple models of these systems and apply general concepts of perturbation in studying their behaviour; this would bring catastrophe theory very close to general control theory.

But one feature common to all these problems, and very correctly Thompson emphasises this, is that they are generally optimal systems. Many are indeed highly optimal. If we try to formulate a general problem it is something on the following lines: as we pursue increasing optimisation in the design and planning of systems, how can we determine in simple terms those parameters to which the stability of the whole system is most sensitive? We might then follow this question with another: is the degree of instability, or sensitivity to the critical parameters, an acceptable one?

Answers to these questions would be invaluable to designers of systems in many areas. The teaching of such concepts as part of the philosophy of design would add a new dimension to our design methodology. The ultimate goal in such studies is to determine common patterns between the many different systems we design and build, and in which catastrophe can occur. What can be envisaged long-term is not only concepts of how to build optimal systems—indeed our knowledge of this is already very extensive—but how to study their sensitivity to disturbances and perturbations, and thus be able—as designers—to distinguish between highly critical and less critical systems.

At the end of the day, of course, catastrophe cannot be avoided, but at least we should have a feel for the sensitivity of any optimal system to catastrophe. Thompson's paper is a helpful pointer in this direction: how can we encourage others in the same general direction?

Strontium isotopes and crustal evolution

from J. Sutton

ONE of the most intriguing problems in geology at the present time is the investigation of the first 2,000 million years of the Earth's history. We have now a detailed knowledge of small areas of crust dating from the latter part of the first 1,000 million years of geological time. But the remnants of crust formed more than 3,500 million years ago are no more than a few tens of kilometres across at the most and are widely separated from one another.

What we badly need is information on large scale activities within the primitive Earth, for this might link our fragmentary scraps of knowledge obtained from the ancient rocks exposed in Minnesota, west Greenland, southern Africa and Antarctica.

Moorbath's paper in this issue of *Nature* (page 395) provides just such information, for the strontium isotope studies he reports here and those which Moorbath, Powell and Taylor have reported elsewhere (*J. geol. Soc. Lond.*, **131**, 213; 1975) bear directly on the question of the movement of material between crust and mantle in Precambrian times. Moorbath reports very low $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the crustal rocks he and his colleagues have investigated and deduces that the rocks in question had formed from material which had been transferred to the crust from the mantle shortly before the times when they last crystallised. He is in effect employing ^{87}Sr as a natural radioactive tracer to follow the circulation of silicates in Precambrian times. This is now a well established technique but Moorbath's application of the method to the very old (roughly 3,700–3,600 Myr) rocks of Greenland and Rhodesia has produced results of

great importance for our understanding of the early Precambrian.

The use of strontium isotopes in this way is in itself a fascinating concept. Since the time the Solar System formed, ^{87}Sr has increased in abundance as ^{87}Rb breaks down, the rate of increase in any rock being governed by the amount of rubidium present. The distribution of strontium isotopes through the Earth is therefore influenced by the manner in which rubidium and strontium are associated in rocks. Since rubidium can be admitted to K^+ sites in the main rock-forming potassium-bearing minerals it is widely distributed as a trace element in the potassium-rich granitic rocks abundant in the upper crust but is much less abundant in the potassium-poor basic and ultrabasic rocks of the lower crust and mantle. Study of the ratio $^{87}\text{Sr}/^{86}\text{Sr}$ can provide critical information as to the origin of an igneous rock. A melt derived from the mantle will be marked by relatively low initial ratios of $^{87}\text{Sr}/^{86}\text{Sr}$ (0.699 from 4,600 million years ago to about 0.704 at the present day). In contrast in continental crust rubidium-rich granite can accumulate ^{87}Sr much more rapidly as the figures illustrating Moorbath's article indicate. Accordingly a melt derived from granites which have resided for long periods of geological time in the crust may inherit this high ratio as an initial feature and so be distinguished from rock derived directly from the mantle. Moorbath has been able to show that the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the 3,700–3,600 million years old Greenland and Rhodesia rocks are so low as to suggest derivation directly from the mantle of those times. He has also

found, as have earlier investigators of rocks formed rather later in the Precambrian, that rocks developed between 2,800 and 2,500 million years ago are marked by low $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. These are close to, or at most a little above, values expected of the mantle at that stage in the Earth's history.

Moorbath concludes that these younger Precambrian rocks, some of which come from Scotland and Greenland, could not have been derived, as has been suggested, by the reworking of very much older granitic crustal rock such as the 3,700 million year old examples of west Greenland. Moorbath follows Hurley in concluding that these facts indicate a progressive addition of mantle material to the crust. He points out in the concluding paragraphs of his article that such transfers of material from mantle to crust seem to have been especially active at certain periods of time during geological history. Some of these periods, it may be noted, coincide with the abrupt changes in continental movement indicated by the hairpins in the apparent polar wander paths established by palaeomagnetic investigations.

One question raised by Moorbath's work with its additional evidence for a near identity of crustal and probable mantle $^{87}\text{Sr}/^{86}\text{Sr}$ ratios through early Precambrian time is of particular interest. Do these results indicate, as Armstrong has proposed, that matter was exchanged more effectively between mantle and crust during the first 2,000 million years? Perhaps at that time no extensive portions of the crust remained isolated for sufficiently long to establish $^{87}\text{Sr}/^{86}\text{Sr}$ ratios significantly higher than those of the mantle.

What came out of Kohoutek's comet?

from J. F. James

A YEAR and a quarter have now passed since Kohoutek's comet passed perihelion with awful anticlimax and if we have learned nothing else, we are now warned once again of the danger of letting empiricism masquerade as science. With the nine months' warning of its approach there was ample time for elaborate international co-operation to be arranged and in spite of the disappointment of the amateurs and the disillusionment of the public the professional astronomers received it with a great broadside of astronomical equipment and studied it more comprehensively than any previous comet.

Many of the more important obser-

vations have been reported in the December 1974 issue of *Icarus* (**23**, No. 4), which was especially dedicated to the comet, and the editors have promised more later on.

The discovery was made by Dr Lubos Kohoutek on March 21, 1973 and from three positions, one from a pre-discovery plate; Dr Brian Marsden published a preliminary orbit on March 26 (IAU Bulletin no. 2514). From its position and magnitude at the time of discovery and from an empirical formula used to predict the future brightness of comets, it was concluded by some astronomers that it would be very bright indeed at perihelion and would become even brighter later on

as the tail developed. In fact it did get to magnitude -2.3 at perihelion and was observed at the time by the crew of Skylab IV, 200 miles above ground. It was of course invisible at ground level because of the glare of sunlight.

Curiously there was no elaborate series of observations from Skylab IV and the observers there used the archaic method of making drawings of the appearance of the comet. These drawings were sent down to Earth by television link. Skylab's crew were the first to observe the tail of the comet and also found the 'anti-tail', a sunward-pointing spike like that of the Arend-Roland comet of 1956. As the

comet drew away from the sun after perihelion passage this spike became less intense, and was only very faintly visible to photographers in mid-January, the time when the comet was most favourably placed for ground-based observations. By then the spike was much attenuated and more like a fan. The spike is thought to be part of the dust-tail of the comet, comprising particles travelling in a different direction from, but in the same line of sight as the head. The dynamics of these spikes are well understood following the theoretical analysis of Finson and Probst some years ago, in *The Astrophysical Journal*.

More remarkably, the Skylab observers noted that the tail near perihelion was bright yellow. It is hard to see how this could be anything other than resonance scattering of sunlight by sodium vapour and the fact that it was bright enough to be seen by the colour-sensitive cells in the retina bears witness to the comet's magnitude at perihelion.

The comet failed to develop a marked dust tail after perihelion passage and this was the cause of all the disappointment. Observers below 40° latitude who knew where to look and what to look for could just see the comet with the naked eye. It was an easy object in 7×50 night glasses, but never spectacular. This did not stop a NASA team (Hyder, Brandt and Roosen) from taking some very fine detailed photographs showing structure in the tail and showing the separation of the dust and gas components.

The chief investigation seems to have been, by common consent, the search for molecules and the analysis of dust in the tail. The central problem—the nature of the head—was not attacked directly, chiefly because the comet was never close enough for any object smaller than about 500 Km across to be resolved. In any case, this is not a problem likely to be solved by anything less than a comet interception probe—a spacecraft designed to 'fly by' at a close distance from the head or even to collide with whatever is at the centre of the head. (This is something that will be well worth doing when a comet comes close enough and enough warning is given to make it feasible.) Thus the contest between the two theories of the nature of the head, the 'dusty snowball' and the 'dense cloud of dust' is no nearer a decision, but the dusty snowball model, the current favourite, received a knock when Traub and Carleton of the Smithsonian Institution searched for and failed to find either water vapour or methane, the chief materials likely to form the 'snow' of the snowball.

Various people found and measured the familiar cometary molecules, such as C₂, CHOH and CN and new tech-

niques were used for some of these observations. For example, Blamont and Festou of CNRS observed OH by resonance scattering, in the infrared, of the lowest electronic transition band of this molecule and HCN was detected by its radio emission in the 3 mm band by a group from Los Alamos, Boulder and Green Bank. More complicated molecules were also observed. For instance, Ulrich and Conklin reported radio emission from methyl cyanide (*Nature*, **248**, 121), and other groups made searches at radio wavelengths. Atoms too were identified by their spectra, sodium and hydrogen being measured by a group from Bell Laboratories and Earlham College, who simultaneously searched for and did not find any trace of helium. Hydrogen emission at Lyman- α (1215.6 Å) was used to photograph the head, both from Skylab and from a rocket-borne camera. This is the brightest of all resonance lines, and showed an enormous halo of about 4° diameter, strongly condensed towards the head of the comet. It was possible to measure the outward velocity of this hydrogen by searching for the moment when its velocity relative to Earth was zero and the light, not shifted by Doppler effect for the moment, was absorbed by the hydrogen in Earth's geocorona. This outward velocity was lower than expected, sufficiently to indicate that the hydrogen was the product of OH dissociation rather than H₂O dissociation.

The dust emitted by the comet received its share of attention but produced nothing startlingly new. E. P. Ney of the University of Minnesota saw once again the 'silicate bump' in the infrared emission spectrum of the dust. In the 8–13 μ m 'window' in the Earth's atmosphere the radiation received from the dust is predominantly its own thermal emission rather than scattered sunlight. The black-body curve is followed except near 10 μ m where there is an excess of intensity. This has been found in the laboratory to be characteristic of metallic silicates, and has been observed in interstellar dust grains as well as in comet tails. Curiously the bump is missing in the spectrum of the anti-tail of Kohoutek's comet.

To sum up: although the public was disappointed the astronomers were not, and if the comet was unremarkable, this was in great contrast to the number of novel and powerful techniques that were used to study it. As in all good investigations, more questions have been raised than answered, and what we all pray for now is a modest comet passing close to earth with plenty of advance warning. Then we might really know what comets are made of and what comes out of them.

Trying to smooth background radiation

from A. C. Fabian

THE two main handles that we have on background radiations are provided by measurements of spectrum and isotropy. Data on these have been well marshalled by students of the microwave background to show that the Universe has passed through some hot, early phase. The other major background radiation occurs at X-ray frequencies, and its origin is still unknown, although it is reasonable to suppose that most of it is extragalactic and originates at cosmological distances. One problem in interpreting observations of small angular scale (about 5°) fluctuations in the X-ray background stems from the point that these fluctuations arise from those sources just not resolved. A glance at the *Uhuru* catalogue of X-ray sources and recent literature shows that a growing number (~30) of extragalactic objects such as clusters of galaxies, radio galaxies and QSOs are detectable by their X-ray emission. This means that the more distant and weaker members will contribute to the fluctuations, and indeed most of the fluctuations will be due to those sources having intensities such that there is about one per detector beam in the sky. They need not necessarily be at cosmological distances, and so the fluctuations may not directly assist in the quest for the source of the X-ray background.

It has been suggested that the fluctuations observed with *Uhuru* are less than would be expected from the counts of sources already detected. This can then be used to suggest that some of the as yet unidentified X-ray sources are really at cosmological distances and that perhaps we are seeing to the edge of the Universe. Other possible interpretations include consideration of the distribution of the sources in the sky, for they may form some crude lattice or at least anti-clump. This means that the formation of one source may inhibit the formation of any other sources nearby.

D. A. Schwartz of the Center for Astrophysics, Cambridge, Massachusetts has posed the question: "Can the constraint of finite mass smooth fluctuations in the background radiation?" (*Astrophysical Journal*, **194**, L139; 1974). The idea here is that the Universe may be considered composed of volume elements each containing an exactly equal finite amount of mass. This mass is then divided between all known objects including X-ray sources. At the extreme, if all the mass goes into X-ray sources the sky must take on a roughly lattice structure and even

if non-X-ray matter is allowed some constraint is still placed upon the X-ray source distribution. Schwartz notes an analogy with the Fano effect, which applies to the number of ion pairs produced by a fast particle in a detector chamber. The distribution of the number of ion pairs is tighter than the Poisson distribution often applied to such problems. He has taken the formulae for this effect over to his work and attempts to show that smoothing is possible and believes that it is not unreasonable that a factor of two smoothing should emerge. No assumptions are made about the distances to sources and it appears that the principle is to be taken quite generally.

What Schwartz has not done is demonstrate that his approach is consistent and that he is justified in using a method from a different branch of physics. Consider a Universe consisting of constant mass elements of some fixed scale size, within which any dispersion of the number of X-ray sources is restricted in some way. Each volume element contains one or more sources but an X-ray detector will observe fluctuations which will be dominated by the distance from the observer where there is about one source per beam. The beam will therefore only observe a small portion of a volume element at one given time, so it matters whereabouts in that element the sources are situated. If they are all in one corner, very large fluctuations will result! The Fano effect does not translate directly to this problem, since the positions of the ion pairs in a detector chamber is irrelevant.

We can conclude therefore that the finite mass constraint does not smooth background radiations to the extent suggested by Schwartz. If the real situation is compared with the artificial one just discussed, it is hard to see that any significant reduction will result at all.

Predators' good housekeeping

from our *Animal Ecology Correspondent*

PREDATORS, in their concern to maintain a supply of food, rarely eat more than a small proportion of the population of their principal prey. Kruuk and Turner (*Mammalia*, **31**, 1; 1967), for example, calculated that lions consumed annually no more than 3% of the wildebeest stocks—their main prey—in the Serengeti. If this did not supply enough food, alternative sources were tapped. Similarly Pearson was able to show that feral domestic cats exerted a powerful pressure on declining rodent populations but switched to alternative

prey when these became too scarce (*J. Anim. Ecol.*, **35**, 217; 1966).

For large predators, such as lions and cats, a switch to alternative prey may be far easier than for small predators which are specifically adapted for feeding on a restricted size range of prey. Brown and Lasiewski have drawn attention to the problems facing weasels in northern tundra regions (*Ecology*, **53**, 939; 1972). Males and females, being of markedly different sizes, exploit different feeding niches and thus function much like two separate species of small predators. Perhaps this size dimorphism makes existence possible in these conditions, or at least permits higher populations. Iverson has pointed out that weasels have a metabolic rate between two and three times higher than that of other mustelids, and a food requirement to match (*J. cell. comp. Physiol.*, **81**, 341; 1972). The ecological implications of an inability to switch to alternative prey coupled with an unusually high food demand are twofold. First, weasels might be expected to be highly territorial with each territory containing sufficient amounts of the correct prey species, and second, they might be expected rapidly to decrease breeding when food becomes too scarce in order to conserve stocks.

Erlinge of Lund has recently published some interesting data on territoriality and population size of the weasel in relation to prey abundance (*Oikos*, **25**, 308; 1974). His study area was 80 ha of spruce plantation, both young and old, deciduous woodland and replanted clearings. Various stone walls and dense cover throughout rendered the whole area a most favourable weasel habitat. Weasels were trapped and marked by ear-clipping during two autumns and one summer. The very high number of recaptures revealed that in the autumn of 1972 the habitat was divided up between three resident territorial males, two females showing evidence of having suckled young, and six young weasels. Three other males visited the area as transients. During the winter of 1972–3 the territorial system was much as it had been the autumn before but a rapid turnover in territory ownership was evidenced by only one of the autumn residents being a territory holder the following summer.

In the autumn of 1972, most weasels had been caught in the spruce plantation. The population of short-tailed voles was then between 30 and 40 per ha. By the following summer the vole density dropped to less than 10 per ha with the result that most of the residents left the plantation and settled in the replanted clearing where the rodent population was some 60% higher. The emigration of all the females from the

study area later in the summer of 1973 meant that there was no breeding.

East and Lockie have made some observations on the food requirements of captive breeding weasels (*Proc. zool. Soc. Lond.*, **143**, 395; 1964). A pregnant female weasel needs 20–30 g of mouse each day for about 35 days, and during the 21 days of lactation she requires about 60 g each day. Once the litter of, say, six young weasels starts eating meat the requirement of food increases over the next 40 days from 69 g to 192 g daily. Translation of these figures into rodent densities requires the acceptance of certain assumptions; namely, that each vole weighs 25 g, that breeding female voles produce 0.125 young each day per adult in the population, that a generation time is 45 days and that the sex ratio is equal. These assumptions accord well with the many published observations on vole productivity. Given them, more than ten reproducing voles are necessary to produce sufficient food for one breeding weasel.

It seems that when the prey density in Erlinge's study sank to less than 10 per ha, breeding was inhibited by the emigration of lone females. Given that the average territory size of females is about 1.5 ha and of males considerably larger, it is hard to see that the reported rodent densities could not support both sexes especially if there is any differentiation in feeding niches. One must conclude that such differentiation is perhaps less clearly defined in rich woodland areas than in the more rigorous northern latitudes. Erlinge's data highlight some of the problems faced by highly specialised, small predators, and some of the difficulties encountered by ecologists in interpreting them.

Noisy meteors

from David W. Hughes

THE sounds associated with bright meteors and fireballs can be divided into two fundamental types—a single or repeated sharp cracking noise and a distinct rumbling, these occurring a few minutes after the passage of the meteoroid—and a second much rarer noise, a hissing sound reported to be heard almost simultaneously with the visual phenomenon.

Theories about the origin, and problems associated with measuring the first type of meteor noise are discussed by ReVelle in the February edition of *Sky and Telescope* (**49**, 87; 1975). He concludes that only fireballs brighter than visual magnitude -8 , with kinetic energies of the order of 10^{15} erg can produce sounds. These penetrate to the lower levels of the atmosphere where

the air is dense enough to act as a fluid and therefore to sustain organised sound waves. One example quoted is the large fireball seen and heard by Wilson at College, Alaska. The meteor at a range of 300 km produced an airwave signal of maximum amplitude 4.6 dyne cm^{-2} which lasted for 12 s. The radius of the strong shock region surrounding the train was estimated to be about 450 m, the incident meteoroid having an energy of $6 \times 10^{20} \text{ erg}$. Assuming a density of 0.3 g cm^{-3} and a geocentric velocity of 11.2 km s^{-1} the meteoroid had a mass of $9 \times 10^9 \text{ g}$ and a diameter of 13 m.

During atmospheric entry the meteoroid can be travelling at 40 to 250 times the speed of sound. The hypersonic flow of air around the meteoroid produces an effect analogous to the explosion wave produced by a cylindrical line source. As the disturbance propagates outward from the meteor it eventually becomes weak enough to be described as a sound wave. The frequency is, however, usually below the audible limit of the human ear and all that can be detected of this infrasonic wave is a sharp 'crack' as it passes due to the rapid pressure change.

The transmission of the sound wave to ground level depends on the temperature, density and wind profiles of the atmosphere. In general the steeper the angle of entry of the meteoroid the less likely it is for a ground observer to hear the sound. Winds in the 40–60 km region can produce channeling effects enabling the sound to propagate over unusually long distances. Atmospheric damping of the wave energy increases with frequency, which accounts for the low frequency rumbling sound analogous to thunder, heard after the passage of the meteoroid. Meteor sounds have characteristic frequencies from about 0.05 to 5 Hz. In general the more energetic the meteoroid the lower the frequency of the sound wave produced.

ReVelle describes how microbarographs are to be used to detect and measure infrasonic sounds from meteors. These are designed to detect pressure amplitude changes in the range 0.1 to 100 dyne cm^{-2} with frequencies between 10^{-3} and 10 Hz and will be installed in the areas of the North America prairies covered by the meteor cameras of the Smithsonian Astrophysical Observatory and the National Research Council of Canada. From known incident fireball fluxes ReVelle expects to record about 10 meteor sounds per microbarograph station during 6 months of night-time observing. Wind noise is one of the major problems but filtering techniques can reduce this to a tolerable level provided the wind speed is less than



THE upper and lower Van Norman dams, which lie in the northern San Fernando Valley about 32 km north-west of Los Angeles, were built between 1912 and 1921 with hydraulic fill placed on young valley alluvium. During the 1971 San Fernando earthquake (magnitude 6.5) both were badly damaged by slope failures on the reservoir embankments. This US Geological Survey picture shows the lower dam just after the earthquake. The 1971 shock was accompanied by the most intense ground motions ever recorded instrumentally for a natural earthquake. But on the basis of geological and historical studies, R. F. Yerkes of the US Geological Survey estimates that earthquakes with magnitudes of at least 7.7 may be expected in this highly populated area. A magnitude 8+ event on the San Andreas fault about 40 km away could also cause extensive damage in the Van Norman area.

10 m.p.h. The recordings will yield energy-against-frequency characteristics of the sound waves and should provide new clues about the meteoroid ablation processes in the lower atmosphere and the complicated interactions which take place at the meteor train-atmosphere boundary.

The second type of noise, the hissing, swishing, buzzing sound heard at the same time or just before the fireball is seen is much more difficult to explain. Such noises have been termed 'anomalous' because they seem to be transmitted at the speed of light. Romig and Lamar in a detailed study of the phenomenon (*Rand Corporation Memorandum RM-3724-ARPA*) have

ruled out the possibilities that the noises are just imagined by the observer (unconsciously associating the fireball with a skyrocket for example) or just chance coincidences. Many trained observers have heard these anomalous sounds and the report contains many examples of people who heard a whizzing noise, looked up and then saw the fireball.

How can this sound be produced? Two other natural phenomena might provide clues. Lightning leader strokes produce brontophonic sounds heard just before the thunderclap from the return stroke. Also aurorae have been reported to produce hissing sounds. Brontophonic noises are produced by

small coronal discharges from surrounding vegetation but it seems very unlikely that enough free charges could be deposited by the fireball passage to explain the noise. The origin of auroral noise is still a mystery.

Considerable radiation must be produced by the fireball in regions of the electromagnetic spectrum other than the visual and this could provide a possible explanation. Electrophonic sounds for example have been heard by people exposed to beams of low powered radar sets. These have been described as buzzing, clicking, hissing or knocking sounds and seem to depend on the transmitter characteristics. An electromagnetic power density as low as $400 \mu\text{W cm}^{-2}$ can be heard, but only by people whose audible hearing by air or bone conduction was good above 5,000 Hz. Perhaps radio frequency electromagnetic waves are acting directly on the brain or inner ear. There are distinct similarities between electrophonic noises and anomalous sounds from fireballs but the production mechanisms of both are still unknown.

With luck the bariophonic recorders will pick up examples of anomalous noise as well as the more normal meteor sounds and help solve this problem also. One snag is that anomalous noise seems to be associated with brighter fireballs (average magnitude -13) which are rare objects indeed.

Plate tectonics and general relativity

from Peter J. Smith

THE mechanism responsible for the movement of lithospheric plates over the Earth's surface is a subject of continuing debate. The majority view is that plate tectonic processes are related to convection in the mantle, although there is less agreement about whether convection is the prime mover or a secondary consequence. If the prime mover, convection would be directly responsible for the motion of the overlying plates, and that of ocean floors in particular; but it is also possible to envisage that plates drift for other reasons (for example, by sliding under gravity) and that 'convection' arises from the need to complete the flow patterns at depth. The latter hypothesis bears some resemblance to the minority view of Van Bemmelen (*Tectonophysics*, 1, 385; 1965) and others, whose 'undation theory' proposes that selective high radioactive heating in the mantle produces warping of the overlying crust followed by lateral spreading under gravity.

But whatever the disagreements over

causes, there is general consent that plate tectonic processes are long term phenomena with characteristic time scales of at least hundreds of millions of years. By contrast, the high seismic activity which defines the locations of plate boundaries is sometimes claimed to have much shorter periodicity of about 11 years (see, for example, Simpson, *Earth Planet. Sci. Lett.*, 3, 417; 1967). Machado (*Geol. Rund.*, 64, 74; 1975) has now drawn attention to another example of this supposed periodicity—in the seismicity of the Azores—which he finds 'informative'. What makes the Azores particularly interesting is that they lie at the triple junction of the American, African and Eurasian plates and are thus presumably influenced both by the constructive margin forming the mid-Atlantic ridge and by the destructive margin along the Eurasian-African boundary. Machado finds that the 11-year variation in earthquake frequency is apparently present in data both from the islands of Fayal and Pico and from the islands of Terceira and San Miguel. But there is a difference in that the main seismic swarms on the two pairs of islands alternate, the main Fayal-Pico swarms being associated with crustal expansion (constructive) and the main Terceira-San Miguel swarms being associated with contraction (destructive).

Machado generalises from this example by suggesting that expansion and contraction perhaps alternate within an 11-year cycle everywhere. He then goes on to propose that this alternation could be due to 11-year pulsations in the gravitational constant (G). Indeed, he does more than propose; he claims to show mathematically that gravitational pulsations are a necessary consequence of general relativity within an expanding Universe. The physical effect of such pulsations would be alternate expansions and contractions not only of the Earth's lithosphere but of the whole interior. During an expansion phase, rifting would take place at oceanic ridges and mantle material would rise to fill the fractures; during the subsequent contraction phase, excess lithosphere would be forced downward at subduction zones. The net effect would be a drift of the plate; but as envisaged by Machado, the mechanism avoids the paradox implicit in attempting to explain apparently simultaneous expansion and contraction by a variation in G . In fact, there is no simultaneous expansion and contraction; each process takes place over alternate 5.5-year half cycles.

It is difficult to know how far to pursue an idea such as this. Is there really an 11-year periodicity in world-wide seismicity? And if so, do world-wide constructive and destructive processes alternate over half-cycles

within each cycle? On the other hand, it would perhaps be unwise to reject the idea of short-period gravitational fluctuations out of hand. The idea of a long term decrease in G has a long and distinguished history; and there is still a lingering suspicion in some quarters that gravitational variations may ultimately be found to account for the Earth's mobility as manifested at the surface by activity at the three types of plate boundary.

Folding proteins along the dotted lines

from Barry Robson

THE relation between the amino acid sequence and conformation of a globular protein is as yet imperfectly understood. But the fact that many proteins can spontaneously fold into their biologically active conformations suggests that computer simulation of the folding process would be likely to throw considerable light on that relation. An interesting technique for carrying out such a simulation has been proposed by Levitt and Warshel in *Nature* (253, 694; 1975).

The work of Levitt and Warshel may be considered as an extension of the study by Ptitsyn and Rashin (Preprint, Acad. Sci. USSR., 1973; *Dokl. Acad. Nauk SSSR*, 213, 473; 1973). Certainly both investigations neglect the polypeptide backbone of the protein, and treat the amino acid side chains essentially as simple single-centred structures with interaction energies calculated from amino acid solubilities in ethanol. In both cases, therefore, emphasis is placed on the hydrophobic interaction between side chains as the principal driving force towards a biologically active conformation. The more recent study, however, is considerably more ambitious. Ptitsyn and Rashin confined their attention solely to the amino acid residues within the extensive α -helical regions of myoglobin, and fixed the conformation of these residues as α -helical from the outset. Levitt and Warshel, on the other hand, propose a technique in which the backbone is free to adopt any conformation.

The problem of manipulating the backbone without having to represent it in detail is overcome by the ingenious use of a device due to the Flory school (P. J. Flory, in *Statistical Mechanics of Chain Molecules*, 248-306, Wiley, New York, 1969). Flory replaced the actual molecular bonds of the backbone by virtual bonds connecting the α -carbon atoms at the base of each side chain. Each virtual bond, represented in the publications of the Flory school as a dotted line, substitutes for one fixed

and two rotatable molecular bonds. Levitt and Warshel propose that the simplified representation of a protein as a set of side chains connected by virtual bonds between their α -carbon atoms is an adequate model for simulating the folding process. In other words, they propose that the computer should literally fold up a protein along the dotted lines.

The authors test this representation by simulating the folding of pancreatic trypsin inhibitor, a small protein of 58 amino acid residues and hence 57 virtual bonds. Since the most stable conformations of a molecule are those of least energy, a procedure is used which minimises the conformational energy as a function of the angles of rotation around the virtual bonds. Unfortunately, the procedure is one which does not allow the folding pathway to cross low energy barriers in the conformational energy surface, barriers which the protein in nature would be expected to negotiate readily. To avoid trapping the molecule in a conformational energy well which is surrounded by low barriers, a technique called 'normal mode thermalisation' is used after each minimisation to generate a new random starting point close to the well. The authors do not, however,

allow for the possibility that during minimisation the simulated pathway of folding could be confined to a trivially shallow channel, a difficulty which could be avoided by the use of a different kind of minimisation procedure. The conformational energy surface of a protein is so complex that the use of certain traditional minimisation procedures may at worst resemble an attempt to plot the potential course of a stream by chasing a free-wheeling locomotive down a railway track. Nevertheless, the authors make the exciting point that in some of their simulations, the protein arrives at a conformation close to that of the known, biologically active structure.

One of the principal difficulties in this study is that the proposed model for a globular protein is such a drastic simplification. As a further check on the validity of their model, the authors confirm that it is apparently stable when set in the biologically active conformation. This is not, however, the sole criterion of an adequate model since the energy surface elsewhere may depart considerably from reality. Another problem is that only very few different starting conformations for the folding simulations were explored, despite the fact that folding even from

the same initial conformation did not necessarily yield the same final conformation. For these reasons there still remains the danger that the approximations to the biologically active conformation obtained by simulated folding are fortuitous, and an artefact of the model and starting conformations chosen.

By far the most serious difficulty, however, is that the use of virtual bonds cannot by its very nature be a general solution to the folding problem. Neglect of the backbone with its capacity for intramolecular hydrogen bonding means that α -helical regions, and possibly other secondary structure features, lose their intrinsic stability. Possible exceptions are those helices with well defined clusters of hydrophobic or hydrophilic side chains on the helix surface, but such helices are by no means ubiquitous. It is therefore notable that Levitt and Warshel, like Ptitsyn and Rashin, were obliged to start with the known α -helical region of trypsin inhibitor in the α -helical conformation and although the virtual bonds within the helical region were allowed to vary, the helix was the part of the real protein reproduced least well after folding. As the authors point out, the various statistical pre-

SHORTLY after the discovery of the first ψ particle with a mass of $3.1 \text{ GeV}/c^2$ (*Phys. Rev. Lett.*, **33**, 1404 and 1406; 1974) the Berkeley-Stanford team, using the e^+e^- storage ring SPEAR at Stanford, California, found a second narrow particle with a mass of $3.7 \text{ GeV}/c^2$ —about four times the mass of the proton (*Phys. Rev. Lett.*, **33**, 1453; 1974). The heavier particle, sometimes called the ψ' (3.7), has also been observed in e^+e^- collisions at the DORIS storage ring near Hamburg (*Phys. Lett.*, **53B**, 489; 1975). The ψ' (3.7) is narrow like the ψ (3.1). Unlike the ψ (3.1) it has not been seen in the collisions of protons, neutrons or γ rays with nuclei, but its rate of production in e^+e^- collisions is only a factor of one third down on the rate for ψ (3.1) production. The ψ' (3.7) decays to e^+e^- , to $\mu^+\mu^-$ and to many-hadron states (probably mostly pions), as does the ψ (3.1). It also decays, about 30% of the time, directly to a ψ (3.1) and two pions. No other narrow states have been reported in the mass region up to about $5 \text{ GeV}/c^2$, though well authenticated rumours from SPEAR tell of a broad bump in the rate of hadron production at a mass of about $4.2 \text{ GeV}/c^2$.

Recent issues of *Physical Review Letters* have been full of theoretical speculation about the meaning of these new objects. Most explanations fall into one of three general groups

The ψ s and their relations

from David J. Miller

—intermediate boson models, 'charm' models or 'colour' models.

The charm or 'SU(4)' model (see *Nature*, **253**, 438; 1974, for a brief outline) is still the most popular. Various schemes have been suggested to fit the ψ and ψ' into a multiplet representation of the group SU(4). In 1961 Gell-Mann and Nishijima succeeded in explaining many of the properties of hadrons by fitting them into multiplets of SU(3)—sometimes called the 'eight-fold way' because many of the multiplets contain eight particles. If the new quantum number charm is allowed, then SU(3) can be extended into SU(4) in a very natural way—just as the previously well established SU(2) scheme of 'isotopic spin' became SU(3) when Gell-Mann added the 'strangeness' quantum number. In a simple SU(4) scheme there is an obvious place for one ψ particle, in the same multiplet as the well established vector mesons ρ , ω and φ , though it is not so easy to fit in two. If the ψ (3.1) is fitted in, then predictions have been made (by M. K. Gailard, Rosner and others) that charmed particles called the F and the D should exist, with masses as low as

$2.2 \text{ GeV}/c^2$. The ψ s could not decay into Fs or Ds, but the Fs and Ds could be produced in pairs with opposite values of the charm quantum number. It has been suggested that the bump at $4.2 \text{ GeV}/c^2$ may be due to the onset of F or D production, but there is no direct evidence to prove this.

A recent paper by a Toronto group (*Phys. Rev. Lett.*, **34**, 541; 1975) suggests a novel way of fitting both the ψ (3.1) and the ψ' (3.7) into the same SU(4) multiplet as the vector mesons. As well as charm, they invoke a new "medium strong" charm-breaking interaction. They assume that the ψ' (3.7) is a combination of a charmed quark and a charmed anti-quark—the obvious assignment for a ψ in the vector meson multiplet. Their new interaction allows them to make the ψ (3.1) from a different mixture of charmed and strange quarks and anti-quarks. As in other SU(4) theories, they predict other new charmed particles which should be seen soon, if they exist; if their scheme is correct the new particles should have extremely interesting properties due to the effects of the medium strong force. Perhaps this force also has something to do with the medium strong properties of the ψ (3.1) in its decay and in photoproduction (see *Nature*, **254**, 180; 1975, for a discussion of these results).

diction rules so frequently applied to the prediction of helical regions could first be utilised in order to locate the α -helices. Nevertheless, the predictions would have to be particularly good because without the capacity for helical hydrogen bonding, the backbone would presumably not be capable of realistically winding or unwinding to compensate for errors in the statistical predictions.

These cautionary notes may well seem pessimistic in the light of future studies but the onus is on the authors to provide a more extensive body of evidence for the validity of their model, particularly evidence resulting from a much larger number of folding simulations. If the technique can be justified it will undoubtedly prove of very great value for folding the non-helical regions of proteins and bringing preset helices together.

The study of Levitt and Warshel reflects the ingenuity and optimism to be found in a number of recent publications relating directly or indirectly to the folding of globular proteins. At meetings and in visits to other laboratories, one can readily detect the underlying tension and enthusiasm; there is an exciting smell of sulphur, carbon, nitrogen and oxygen in the air.

Michael Levitt and Arieh Warshel reply—ROBSON sees our work as similar to that published by Ptitsyn and Rashin (*Dokl. Akad. Nauk. SSSR*, **213**, 473; 1973). Ptitsyn and Rashin have made a physical model of myoglobin out of nine preformed helical segments connected by pieces of flexible chain. They used regular plasticine cylinders for the helices (rather like the simplified models made by protein crystallographers from low resolution electron density maps), and manually searched possible types of packing after making some assumptions about the neighbouring helices along the chain coming together first. The interaction energy of a pair of helices was obtained by counting which residues were taken out of water at the contact surface, hydrophobic residues preferring to be buried and hydrophilic residues to be exposed. With this model they concluded that an arrangement of helices closely like native myoglobin had both the lowest energy and the most stable intermediates at each stage of the assembly. We find Ptitsyn and Rashin's work exciting because of its originality and the unexpectedly clear-cut picture of stable subassemblies when forming myoglobin from helices. It is surprising that their work is considered so similar to what we have done. Our work is a complete *ab initio* computer simulation of the folding of a whole protein chain based on realistic chain geometry

and interatomic forces derived from studies of small molecules, and including both the dynamics of the chain and randomising effect of temperature. In our paper (*Nature*, **253**, 694; 1975), we did not neglect the polypeptide backbone but only omitted the hydrogen bonds between peptides which may be weak in the early stages of folding when the protein has a fairly open conformation in an aqueous environment. We said they could be introduced later, along with other detailed interactions. It is certainly not true that we are "obliged to start with the known α -helical region of trypsin inhibitor" (see page 697, column 2 of our article). We include the effect of the solvent using amino acid solubilities but only as part of a realistic energy function with contributions from van der Waals, electrostatic, and torsional forces.

We did, of course, refer to Flory's work on the virtual bond approximation, which was designed and used for calculating statistical properties of disordered random coil polymers. It was totally unexpected that this simplification could also be used in a complete simulation of protein folding. It now seems that the protein chain can be simplified because the peptide group is planar and the two rotatable bonds almost co-linear. Each value of our single variable torsion angle α replaces a pair of the usual backbone torsion angles ϕ and ψ , so that we can halve the number of variables and hardly affect the representation of the protein backbone. It is unfair to say that we consider the protein as "side chains connected by virtual bonds". We connect adjacent C's by a rotatable virtual bond and the side chains then stick out from this backbone (see Fig. 1). Omitting the backbone and connecting adjacent side chains by a virtual bond would be a very poor model indeed.

We do not believe that the native conformation of a protein necessarily has the lowest free energy, but try to simulate the actual folding process. For this one must move smoothly over the energy surface down the energy gradient. The minimisation method we use, developed by the Numerical Analysis group at Harwell, has been proven to be very powerful for a wide class of problems; it follows the path down the gradient more efficiently than any other method. We are well aware of the so-called "untraditional" non-gradient minimisation methods which could conceivably avoid shallow minima, but these would require between 50 to 100 times as many energy evaluations to reach a minimum. What would be worse is that one would lose all physical reality and still have no guarantee of crossing low energy barriers. Our normal mode thermalisation

technique uses information gathered at no extra cost while dropping into a minimum to get out again. In fact very few shallow minima are encountered (usually about 1 or 2, see Fig. 4) in folding from an open to compact conformation. This is an expected result of averaging over less important detail, but may well represent, as we wrote, a real property of the first stage of the actual folding process. The conformational energy surface of a protein seems rather simple to us, and the analogy of following a freewheeling locomotive is actually a close picture of what we find.

Until now nobody has even shown that accepted interatomic forces give rise to a true minimum (net couples less than 10^{-6} kcal mol $^{-1}$ rad $^{-1}$) near the native conformation of a protein. Were we to have used the accepted all-atom representation of pancreatic trypsin inhibitor, our work would have cost more than NASA's Apollo programme, and the results would have been much harder to interpret because of the many shallow minima caused by the minor irregularities of the side chains. Throughout this project we have been very aware of the necessity of making the calculations as efficient as possible; we have managed to reduce the calculation time by a factor of about 1,000,000 by (1) considering the most effective variables, (2) averaging over side-chain conformations, (3) using a very efficient minimisation method, and (4) using normal modes in the thermalisation.

Since last August when we obtained the results published in *Nature*, we have extended the work considerably. We have simulated pancreatic trypsin inhibitor folding including the backbone hydrogen bonds and get as good a fit to the native conformation. We have also tested different starting conformations and sets of energy parameters, and still obtain a significant success rate. The same methods have been applied to rubredoxin, another small protein, with encouraging results. Tests on the "mainly helical" proteins myoglobin and myogen have shown that when peptide hydrogen bonds are included, helices are stable and capable of growth and that several helices can come together as in the native protein. Now that we have a new version of the program two and a half times faster (one cycle for PTI in 0.2 s) we will look at bigger proteins, like lysozyme, ribonuclease, and the antibody domains.

For the first time it is possible to simulate protein folding by computer, study each step of the process in detail, and test which forces dominate and which parts fold first. A great deal still has to be done, but there is room for some optimism about a general solution to the problem of how proteins fold.

review article

Bacterial behaviour

Howard C. Berg*

Bacteria swim by rotating their flagella. They back up or choose new directions at random by changing the direction of the rotation. The probability of such changes is biased by sensory reception. The bias depends on the way in which the intensity of the stimulus changes with time, so that the bacteria tend to swim up a gradient of attractant and down a gradient of repellent chemicals.

BACTERIA move with intent, accumulating in regions which are hot or cold, light or dark, or of favourable chemical content. Systematic studies of these phenomena began in the late nineteenth century¹⁻³, but only recently has it been clear that they can be understood in molecular detail⁴. How do bacteria swim? How do they respond to changes in their environment? How do they detect and process sensory information?

Mechanism of propulsion

Bacteria swim by rotating thin helical filaments which project from one or more points on their surface (Fig. 1). A filament is joined by a proximal hook to a rod and a set of rings embedded in the cell wall and the cytoplasmic membrane⁵. The entire structure is called a flagellum. If there are several flagella per cell, the filaments form bundles and move in unison. The bundle both pushes (or pulls) and rotates the cell (Fig. 1). Bütschli⁶ realised long ago that this is a natural consequence of the resistance of the medium to the motion of the flagella. Whether a flagellum bends in a helical fashion, as in some eukaryotes, or rotates rigidly about its axis, as in bacteria, the effect is the same. The forces involved are viscous rather than inertial⁷⁻⁸, so much so that it is impossible for a bacterium to coast: when the flagella stop moving, the cell comes to a standstill (in so far as Brownian motion will allow) within about a millionth of a body length. The hydrodynamics of the propulsion of cells with polar flagella are well understood⁹⁻¹¹.

Bacterial and eukaryotic flagella are entirely different organelles. The eukaryotic flagellum contains complex bending machinery^{12,13}, the bacterial flagellum does not¹⁴⁻¹⁷. Its filament is only about 130 Å in diameter. It is a self-assembling polymer of a single protein, flagellin, which has no known enzymatic activity. Since the filament lacks local means for the interconversion of chemical and mechanical energy, it cannot actively bend. How, then, does it do work on the medium in which it is suspended? It has been suggested that helical waves are driven from the base^{16,18-20}, but it is not clear that a filament propagating such waves could transfer the requisite amount of energy. If the filament rotates as a whole, energy transfer is no longer a problem. Rigid rotation was noted as a possibility by Stocker¹⁴, proposed^{21,22} and later abandoned^{23,24} by Doetsch, then adopted²⁵⁻²⁷ and later modified²⁸ by Jarosch. Unfortunately, one cannot distinguish wave propagation from rigid rotation simply by looking at a cell²⁹. This is easily demonstrated with a model of a flagellum made by threading a rubber tube over a spiral wire³⁰. If the tube is fixed at one end and the wire is turned, the tube propagates a helical wave. It looks as if it is rotating, but it is not. If the wire is held fixed and the tube is turned, the tube revolves around the wire. It looks as if

it is stationary, but it is not. Unless one is close enough to see what is happening at the surface of the tube, it is impossible to tell that the tube is there.

There is now a considerable body of evidence in favour of rigid rotation^{31,32}. The most dramatic has been obtained by tethering a cell with an abnormally long hook or a straight filament to a glass slide with anti-hook or anti-filament antibodies; when this is done, the cell body rotates, alternately clockwise and counter-clockwise³². The cell is non-motile when free, but it spins several revolutions per second when the hook or the filament is linked to the glass. Similar results have been obtained with anti-filament antibodies and cells with helical filaments³³. In each case, the body of the cell rotates; it does not merely precess or wobble. It has been suggested that tethered cells may rotate because the end of the hook or filament in contact with the glass crawls around a circular path as it passes a helical wave (C. R. Calladine, personal communication). Experiments with polystyrene latex beads³² are not subject to this argument. When they are linked to cells with straight filaments, often several can be seen in tangential contact with (and revolving in synchrony about) a line projecting from the surface of the cell

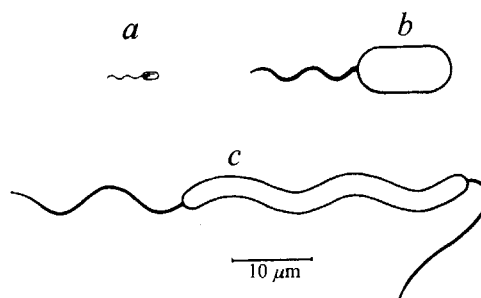


Fig. 1 Scale drawings of various bacteria. *a*, *E. coli* (or *Salmonella typhimurium*). About six filaments arise at random from the sides of the cell and form a bundle which appears near one pole. The bundle pushes the cell. When it changes its orientation, the cell goes off in a new direction. *b*, *Chromatium okenii*. About forty filaments arise at one pole. When they change their direction of rotation, the cell backs up. *c*, *Spirillum volutans*, shown swimming from left to right. The body is helical. About twenty-five filaments arise at each pole. Those on the right are in the head configuration, those on the left in the tail configuration. When they change their direction of rotation and flip over, the cell swims in the opposite direction. In each case, the filaments rotate about 50 revolutions per second one way while the body of the cell rotates more slowly the other; translation occurs at speeds of about 20 cell diameters a second.

*Address: Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80302.

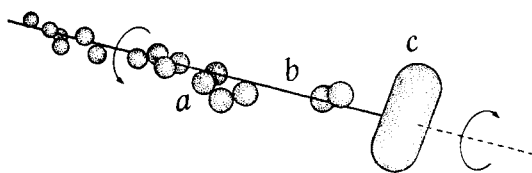


Fig. 2 An experiment in which polystyrene latex beads (a) are linked to a straight filament (b). The filament is shown as a solid line, but it is invisible under phase contrast. Most of the beads are linked directly to the filament; some are linked by means of other beads. They revolve about the axis of the filament in one direction, while the cell body (c) rotates about this axis in the other. There are no beats or undulations. The beads remain fixed relative to one another. Every now and again they slow down, stop, and start up in the opposite direction; the cell body changes its direction synchronously. The entire assembly slowly diffuses through the medium. The cell was derived from *E. coli* AW405 (ref. 34) by transduction with P1 from the donor W3110 (ref. 32). The beads were linked to the filament by a method similar to that of Silverman and Simon³².

(Fig. 2). The flagella do not simply wind up and unwind: mutants exist which rotate in one direction indefinitely³³.

If the flagella rotate, it is possible to understand (see ref. 31) how cells with flagellar bundles are stopped by bivalent but not by univalent anti-filament antibodies^{35,36}, why cells with only one filament are inert to such bivalent antibodies³⁶, how peritrichously flagellated cells are stopped by flagellotropic phages^{37,38}, how cells with straight filaments may be able to form flagellar bundles³⁹, and finally, how cells with polyhooks but no filaments can counter-rotate when linked together with bivalent anti-hook antibodies⁴⁰. In making the first of these arguments, we noted that adjacent filaments in a bundle will stop when crosslinked by an antibody, because they can no longer rotate relative to one another. Calladine⁴¹ has argued that these crosslinks, if sufficiently rigid, could also prevent helical wave propagation by suppressing intra-filament "oscillatory lengthwise rubbing". This assumes that the filaments are inelastic, when, in fact, relatively little work would have to be done to deform them to meet the constraints on wave motion which such crosslinks impose (R. A. Anderson, personal communication).

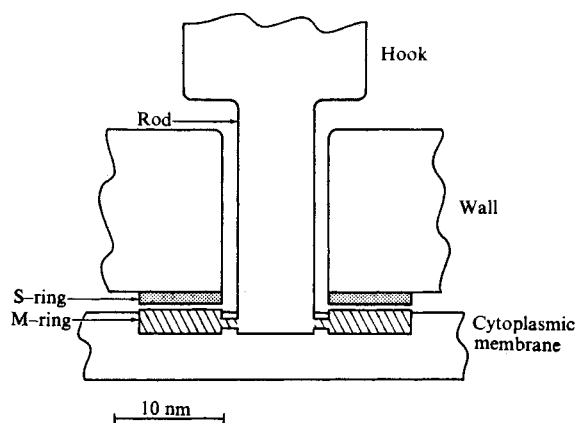


Fig. 3 A model for the flagellar rotary motor of a Gram-positive bacterium. The rod is connected to the filament (not shown) by the hook, which serves as a universal joint⁴¹. The torque is generated between the M-ring, which is mounted rigidly on the rod, and the S-ring, which is mounted on the wall. The M-ring rotates freely in the cytoplasmic membrane. The motor of a Gram-negative bacterium has an additional pair of rings⁵ which probably serve as a bushing for the passage of the rod through the multi-layered wall⁴².

A model for the flagellar rotary motor is shown in Fig. 3 (see ref. 42). The motor utilises as an energy source an intermediate in oxidative phosphorylation rather than ATP itself^{43,44}. It shares this property with a number of systems for active transport⁴⁵. Indeed, it could be driven by the translocation of ions through the M-ring which interact with fixed charges on the surface of the S-ring. Some of the dynamic properties of the motor have been determined by following the rotation of tethered cells in the tracking microscope⁴². The motor runs in either direction at about the same speed, it changes its direction abruptly, the motion can be remarkably smooth, there is little passive slip between the flagellum and the cell wall, and the amount of torque generated is impressive. In *Escherichia coli*, some twenty genes are required for the complete assembly and function of the organelle^{46,47}; many are homologous to those found in *Salmonella*^{48,49}. One (*hag*) is the structural gene for flagellin, another (*flaE*) controls the length of the hook, a third (*flaI*) regulates the synthesis of the entire structure. A normal *mot* product is required for the motor to rotate; normal *flaA* and *cheA* products are required for it to rotate both clockwise and counter-clockwise. Most of the other genes have a non-flagellated mutant phenotype. Only the *hag* gene product has been identified.

Response to changes in environment

Bacteria have a limited repertoire of movements which vary depending on the size and shape of the cell and the distribution of the flagella (Fig. 1). Most swim steadily in almost a straight line, then alter course abruptly. *E. coli* chooses a new direction

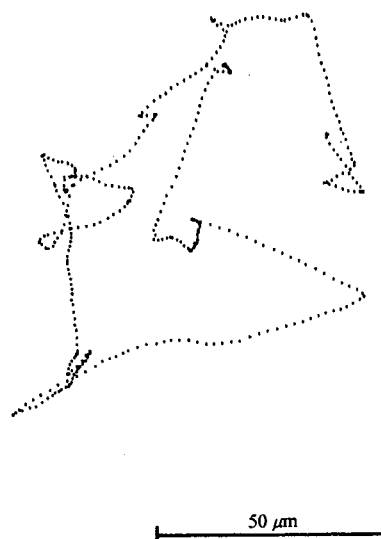


Fig. 4 A projection of the track of a wild type *E. coli* obtained with a microscope which automatically follows its motion in three dimensions^{50,51}. The points are 0.08 s apart. The cell swims along as straight a path as rotational diffusion will allow (runs), stops and jiggles about (tumbles or twiddles), and then runs again. The twiddles occur at random, on the average about once a second.

almost at random (Fig. 4)⁵⁰. *Chromatium okenii* backs up, moves bundle-first several body lengths, then swims forward once again⁵², a manoeuvre which Engelmann⁵³ called a "shock reaction". Cells with a single polar flagellum, such as *Vibrio metchnikovii* (not shown) simply back up; they swim equally well with the flagellum in front or behind⁵⁴. *Spirillum volutans* flips its bundles from a head-tail to a tail-head configuration and swims off in the opposite direction^{54,55}. The probability of the occurrence of these events is biased by sensory reception. It was thought for many years that bacteria back up or choose new directions at random on entering regions which are unfavourable, that is, that the motor reflex is an avoidance response^{53,56,57}. This has not proved to be the case for *E. coli*⁵⁰. When these

bacteria swim up a spatial gradient of an attractant (such as L-aspartate or L-serine), the probability that a twiddle will occur is smaller than it is in an isotropic solution; when they swim down such a gradient, the probability is nearly the same as it is in an isotropic solution. Other parameters of the random walk remain constant. The bacteria drift up the gradient because the runs are somewhat longer in the favourable direction. The same asymmetry has been found in experiments in which a temporal rather than a spatial gradient is formed by enzymatic generation or destruction of an attractant. As the concentration increases, the bacteria change direction less frequently; as it decreases, they swim as they do in the absence of a stimulus⁵⁸. Similar conclusions have been reached in experiments with *S. typhimurium* in which solutions of an attractant at different concentrations are mixed^{59,60}. If the concentration of the attractant is suddenly increased, the bacteria swim smoothly for a relatively long period of time. If it is suddenly decreased, they twiddle more frequently, but only for a relatively short period of time. Attractants and repellents have opposite effects⁶¹. The enzyme and the mixing experiments both show that the bacteria make temporal rather than spatial comparisons⁵⁶. In the enzyme experiments a response occurs even though the medium is isotropic. In the mixing experiments the bacteria are exposed to both temporal and spatial inhomogeneities; they could measure the latter and time-average the results, but then the response would be the same on adding or diluting out the attractant.

The motor reflex occurs when the flagellar 'motors' change their direction of rotation. This was shown in early work on bacteria with polar flagella by Reichert⁶⁴, Buder⁵², and Metzner⁶⁶ who, however, regarded the change as one in the direction of propagation of flagellar waves. The use of the tethering technique has recently made a more direct proof possible for *E. coli*. Larsen *et al.*³³ have shown that mutants of *E. coli* that swim smoothly (that do not twiddle) rotate counter-clockwise when tethered to the top of a slide and viewed from above, while mutants that twiddle incessantly rotate clockwise. Stimuli which cause the wild type to swim smoothly (or to twiddle) cause such cells, when tethered, to rotate counter-clockwise (or clockwise). Similar conclusions have been drawn from studies of *S. typhimurium*⁶². The bundle of a peritrichously-flagellated bacterium is able to push the cell but not to pull it; when the motors reverse, the bundle changes its orientation or comes apart^{54,63,64}, and the cell twiddles³³. The bundle of a bacterium with a polar flagellum is not subject to this instability; it remains intact, and the cell backs up. *Chromatium okenii* completes the shock reaction by swimming forward once again, probably when its filaments tangle; a similar mechanism may terminate the twiddle^{3,19}. In so far as all bacteria able to respond to sensory stimuli are known to change directions spontaneously, it is likely that all bacteria have a "twiddle generator"⁶⁰ that reverses the flagellar motors³. If so, the behaviour of a cell depends on the rate at which the generator fires in the absence of an external stimulus and on the extent to which the firing is enhanced or suppressed by sensory reception. Parkinson⁶⁵ has concluded from an analysis of a large number of motile but non-chemotactic mutants of *E. coli* that the machinery for twiddle generation and suppression involves only three gene products. S-adenosyl methionine may be required for its function^{4,66-68}.

Chemoreception

A renaissance occurred in 1969 when Adler⁶⁹ proved that bacteria have specific chemoreceptors, that is, that they sense attractants *per se*. There were suggestions of this in the early literature (for example, in Rothert's⁵⁶ experiments on competitive inhibition), but the idea never took hold. Links⁷⁰, for example, proposed that the first (or only) step in the chain of events leading to the chemotactic response is a sudden decrease in the rate of utilisation of energy by the motor apparatus. This hypothesis was developed further by Clayton^{71,72}, who applied it to taxis in *Rhodospirillum rubrum*. Adler grew *E. coli* on a chemically defined medium, washed and suspended the cells in a medium that supported motility but not growth⁷³, and then exposed the

cells to spatial gradients formed by the diffusion of attractants, from capillary tubes⁷⁴⁻⁷⁶. By varying the initial concentrations of the attractant and counting the numbers of bacteria that swam into the tubes in a given period of time, he was able to establish a quantitative dose-response curve. Using this method he showed that (1) chemicals that are extensively metabolised need not attract (even if they are the first metabolic products of chemicals that do attract); (2) chemicals that are not metabolised (because they are non-metabolisable *per se* or because the cells have lost the ability to metabolise them) may attract; (3) the response to an attractant is not generally blocked by the presence of structurally unrelated compounds (even if metabolisable); (4) structurally related compounds compete; (5) mutants exist which lack specific taxes (yet metabolise the attractant); and (6) transport of a chemical into the cell is neither necessary nor sufficient for it to attract.

Chemoreceptors for amino acids⁷⁷, sugars⁷⁸, and a number of repellents^{61,79} are now known. The recognition components for D-galactose, D-ribose and maltose are shockable binding proteins⁸⁰. The role of the galactose binding protein in chemotaxis has been studied by Hazelbauer and Adler⁸⁰, that of the ribose binding protein by Aksamit and Koshland^{81,82}, and that of the maltose binding protein by Hazelbauer⁸³. Genetic analysis has shown that the binding protein for galactose interacts with other components that are specific either for taxis or for transport⁸⁴. The recognition components for D-glucose, D-fructose and D-mannitol are sugar-specific enzymes of the phosphotransferase system^{78,85}. Other components in these systems specific for taxis but not for transport remain to be identified. For L-aspartate and D-galactose, twiddle suppression depends on the time rate of change of the fractional amount of chemoreceptor bound^{58,86}. For D-ribose the duration of the smooth response in a mixing experiment is proportional to the increment in the fractional amount of chemoreceptor bound⁸⁷. The cells monitor the status of their chemoreceptors, but the biochemical mechanisms are not known.

Signal processing

Neurons integrate excitatory and inhibitory inputs electrically, responding in an all-or-none fashion by generating an action potential. It is tempting to think of a bacterium in the same manner⁸⁸ and to suppose that the integration of different inputs^{61,69} and the coupling between the receptors and the flagella occur through changes in the membrane potential⁹⁰. This is suggested, for example, by work on *S. volutans* (Fig. 1). Ordinarily, the flagellar bundles reverse synchronously⁶⁵; they seem to do so even when filmed at 48 frames per second⁹¹. This implies that one end of the cell can signal the other in 10⁻² s or less. If the signalling were done by diffusion of a substance of low molecular weight, either through the cytoplasm or along the membrane, at least 1 s would be required³. An electrical disturbance could be propagated much more rapidly. The signalling can be impeded by the addition of large amounts of salt or blocked by partial deprivation of oxygen in the presence of a photodynamic dye⁵⁵. Other substances put the flagella in a head-head or a tail-tail configuration^{66,91,92}, but they may do so simply by forcing the motors to run in the same direction. Some work has been done with drugs known to affect excitable membranes⁹¹⁻⁹⁵, but the results are hard to interpret. In *E. coli* relatively little is known about the mode of coupling between the chemoreceptors and the flagella^{4,47}. Mutants exist that fail to respond to compounds that use different chemoreceptors, such as D-ribose and D-galactose⁸⁴, or L-serine and certain repellents⁷⁹; L-aspartate and L-serine signal the flagella by means of different pathways⁶⁶. The system may prove to be complex. Since both the receptors and the flagella reside in or interact with the cytoplasmic membrane, the latter's involvement is apparent.

This work was supported by grants from the US National Science Foundation and the US National Eye Institute.

¹ Weibull, C., in *The Bacteria*, 1 (edit. by Gunsalus, I. C., and Stanier, R. Y.), 153-205 (Academic, New York, 1960).
Ziegler, H., *Handb. Pfl. Physiol.*, 17(2), 484-532 (1962).

- ³ Berg, H. C., *A. Rev. Biophys. Bioeng.* (in the press).
- ⁴ Adler, J., *A. Rev. Biochem.* (in the press).
- ⁵ DePamphilis, M. L., and Adler, J., *J. Bact.*, **105**, 384-395 (1971).
- ⁶ Bütschli, O., *Klassen und Ordnungen des Thier-reichs*, **1**(2), 856-857 (Winter, Leipzig, 1884).
- ⁷ Ludwig, W., *Z. vergl. Physiol.*, **13**, 397-504 (1930).
- ⁸ Taylor, G., *Proc. R. Soc.*, **A209**, 447-461 (1951).
- ⁹ Chwang, A. T., and Wu, T. Y., *Proc. R. Soc.*, **B178**, 327-346 (1971).
- ¹⁰ Schreiner, K. E., *J. Biomech.*, **4**, 73-83 (1971).
- ¹¹ Coakley, C. J., and Holwill, M. E. J., *J. theor. Biol.*, **35**, 525-542 (1972).
- ¹² Sleight, M. A. (ed.), *Cilia and Flagella* (Academic, London, 1974).
- ¹³ Satir, P., *Scient. Am.*, **231**, 45-52 (1974).
- ¹⁴ Stocker, B. A. D., *Symp. Soc. gen. Microbiol.*, **6**, 19-40 (1956).
- ¹⁵ Iino, T., *Bacteriol. Rev.*, **33**, 454-475 (1969).
- ¹⁶ Asakura, S., *Advan. Biophys.*, **1**, 99-155 (1970).
- ¹⁷ Smith, R. W., and Koffler, H., *Advan. Microbiol. Physiol.*, **6**, 219-339 (1971).
- ¹⁸ Klug, A., *Symp. int. Soc. Cell Biol.*, **6**, 1-18 (1967).
- ¹⁹ Lowy, J., and Spencer, M., *Symp. Soc. exp. Biol.*, **22**, 215-236 (1968).
- ²⁰ Harris, W. F., and Scriven, L. E., *J. Mechanochem. Cell Motility*, **1**, 33-40 (1972).
- ²¹ Doetsch, R. N., *J. theor. Biol.*, **11**, 411-417 (1966).
- ²² Doetsch, R. N., and Hageage, G. J., *Biol. Rev.*, **43**, 317-362 (1968).
- ²³ Valtuzis, Z., and Doetsch, R. N., *J. Bact.*, **100**, 512-521 (1969).
- ²⁴ Doetsch, R. N., *CRC Crit. Rev. Microbiol.*, **1**, 73-103 (1971).
- ²⁵ Jarosch, R., *Osterr. bot. Z.*, **114**, 255-306 (1967).
- ²⁶ Jarosch, R., *Mikroskopie*, **25**, 186-196 (1969).
- ²⁷ Jarosch, R., *Acta Protozool.*, **11**, 23-38 (1972).
- ²⁸ Mussill, M., and Jarosch, R., *Protoplasma*, **75**, 465-469 (1972).
- ²⁹ Harris, W. F., *Protoplasma*, **77**, 477-479 (1973).
- ³⁰ Taylor, G., *Proc. R. Soc.*, **A211**, 225-239 (1952).
- ³¹ Berg, H. C., and Anderson, R. A., *Nature*, **245**, 380-382 (1973).
- ³² Silverman, M., and Simon, M., *Nature*, **249**, 73-74 (1974).
- ³³ Larsen, S. H., Reader, R. W., Kort, E. N., Tso, W.-W., and Adler, J., *Nature*, **249**, 74-77 (1974).
- ³⁴ Armstrong, J. B., Adler, J., and Dahl, M. M., *J. Bact.*, **93**, 390-398 (1967).
- ³⁵ Greenbury, C. L., and Moore, D. H., *Immunology*, **11**, 617-625 (1966).
- ³⁶ DiPierro, J. M., and Doetsch, R. N., *Can. J. Microbiol.*, **14**, 487-489 (1968).
- ³⁷ Meynell, E. W., *J. gen. Microbiol.*, **25**, 253-290 (1961).
- ³⁸ Raimondo, L. M., Lundh, N. P., and Martinez, R. J., *J. Virol.*, **2**, 256-264 (1968).
- ³⁹ Iino, T., and Mitani, M., *J. gen. Microbiol.*, **49**, 81-88 (1967).
- ⁴⁰ Silverman, M. R., and Simon, M. I., *J. Bact.*, **112**, 986-993 (1972).
- ⁴¹ Calladine, C. R., *Nature*, **249**, 385 (1974).
- ⁴² Berg, H. C., *Nature*, **249**, 77-79 (1974).
- ⁴³ Larsen, S. H., Adler, J., Gargus, J. J., and Hogg, R. W., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1239-1243 (1974).
- ⁴⁴ Thipayathasana, P., and Valentine, R. C., *Biochim. biophys. Acta*, **347**, 464-468 (1974).
- ⁴⁵ Boos, W., *A. Rev. Biochem.*, **43**, 123-146 (1974).
- ⁴⁶ Hilmen, M., Silverman, M., and Simon, M., *J. supramol. Struct.*, **2**, 360-371 (1974).
- ⁴⁷ Parkinson, J. S., *Cell*, **4**, 183-188 (1975).
- ⁴⁸ Yamaguchi, S., Iino, T., Horiguchi, T., and Ohta, K., *J. gen. Microbiol.*, **70**, 59-75 (1972).
- ⁴⁹ Vary, P. S., and Stocker, B. A. D., *Genetics*, **73**, 229-245 (1973).
- ⁵⁰ Berg, H. C., and Brown, D. A., *Nature*, **239**, 500-504 (1972).
- ⁵¹ Berg, H. C., *Rev. scient. Instrum.*, **42**, 868-871 (1971).
- ⁵² Buder, J., *Jahrb. wiss. Bot.*, **56**, 529-584 (1915).
- ⁵³ Engelmann, T. W., *Pflügers Archs ges. Physiol.*, **30**, 95-124 (1883).
- ⁵⁴ Reichert, K., *Zentbl. Bakt. Parasitenk. Abt. I. Orig.*, **51**, 14-94 (1909).
- ⁵⁵ Metzner, P., *Jahrb. wiss. Bot.*, **59**, 325-412 (1920).
- ⁵⁶ Rothert, W., *Flora*, **88**, 371-421 (1901).
- ⁵⁷ Jennings, H. S., and Crosby, J. H., *Am. J. Physiol.*, **6**, 31-37 (1901).
- ⁵⁸ Brown, D. A., and Berg, H. C., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1388-1392 (1974).
- ⁵⁹ Macnab, R., and Koshland, D. E., Jr., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2509-2512 (1972).
- ⁶⁰ Koshland, D. E., Jr., *FEBS Lett.*, **40**, S3-S9 (1974).
- ⁶¹ Tsang, N., Macnab, R., and Koshland, D. E., Jr., *Science*, **181**, 60-63 (1973).
- ⁶² Taylor, B. L., and Koshland, D. E., Jr., *J. Bacteriol.*, **119**, 640-642 (1974).
- ⁶³ Pijper, A., *J. path. Bact.*, **47**, 1-17 (1938).
- ⁶⁴ Macnab, R., and Koshland, D. E., Jr., *J. molec. Biol.*, **84**, 399-406 (1974).
- ⁶⁵ Parkinson, J. S., *Nature*, **252**, 317-319 (1974).
- ⁶⁶ Armstrong, J. B., *Can. J. Microbiol.*, **18**, 591-596 (1972).
- ⁶⁷ Armstrong, J. B., *Can. J. Microbiol.*, **18**, 1695-1701 (1972).
- ⁶⁸ Aswad, D., and Koshland, D. E., Jr., *J. Bact.*, **118**, 640-645 (1974).
- ⁶⁹ Adler, J., *Science*, **166**, 1588-1597 (1969).
- ⁷⁰ Links, J., thesis, Rijksuniversiteit, Leiden (1955).
- ⁷¹ Clayton, R. K., *Handb. Pfl. Physiol.*, **17**(1), 371-387 (1959).
- ⁷² Clayton, R. K., in *Photophysiology*, **2** (edit. by Giese, A. C.) 51-77 (Academic, New York, 1964).
- ⁷³ Adler, J., and Templeton, B., *J. gen. Microbiol.*, **46**, 175-184 (1967).
- ⁷⁴ Pfeffer, W., *Unters. Bot. Inst. Tübingen*, **1**, 363-482 (1884).
- ⁷⁵ Pfeffer, W., *Unters. Bot. Inst. Tübingen*, **2**, 582-663 (1888).
- ⁷⁶ Adler, J., *J. gen. Microbiol.*, **74**, 77-91 (1974).
- ⁷⁷ Mesibov, R., and Adler, J., *J. Bact.*, **112**, 315-326 (1972).
- ⁷⁸ Adler, J., Hazelbauer, G. L., and Dahl, M. M., *J. Bact.*, **115**, 824-847 (1973).
- ⁷⁹ Tso, W.-W., and Adler, J., *J. Bact.*, **118**, 560-576 (1974).
- ⁸⁰ Hazelbauer, G. L., and Adler, J., *Nature new Biol.*, **230**, 101-104 (1971).
- ⁸¹ Aksamit, R., and Koshland, D. E., Jr., *Biochem. biophys. Res. Commun.*, **48**, 1348-1353 (1972).
- ⁸² Aksamit, R. R., and Koshland, D. E., Jr., *Biochemistry*, **13**, 4473-4478 (1974).
- ⁸³ Hazelbauer, G. L., *J. Bact.* (in the press).
- ⁸⁴ Ordal, G. W., and Adler, J., *J. Bact.*, **117**, 517-526 (1974).
- ⁸⁵ Adler, J., and Epstein, W., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2895-2899 (1974).
- ⁸⁶ Mesibov, R., Ordal, G. W., and Adler, J., *J. gen. Physiol.*, **62**, 203-223 (1973).
- ⁸⁷ Spudich, J., and Koshland, D. E., Jr., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ⁸⁸ Clayton, R. K., *Archs Mikrobiol.*, **19**, 141-165 (1953).
- ⁸⁹ Adler, J., and Tso, W.-W., *Science*, **184**, 1292-1294 (1974).
- ⁹⁰ Doetsch, R. N., *J. theor. Biol.*, **35**, 55-66 (1972).
- ⁹¹ Krieg, N. R., Tomeity, J. P., and Wells, J. S., Jr., *J. Bact.*, **94**, 1431-1436 (1967).
- ⁹² Caraway, B. H., and Krieg, N. R., *Can. J. Microbiol.*, **18**, 1749-1759 (1972).
- ⁹³ Clayton, R. K., *Archs Mikrobiol.*, **29**, 189-212 (1958).
- ⁹⁴ Faust, M. A., and Doetsch, R. N., *Can. J. Microbiol.*, **17**, 183-189 (1971).
- ⁹⁵ Faust, M. A., and Doetsch, R. N., *Can. J. Microbiol.*, **17**, 191-196 (1971).

articles

Experiments in catastrophe

J. M. T. Thompson

Department of Civil Engineering, University College, London, UK

Two new theories of evolutionary instability are outlined. When applied to highly optimised engineering structures, they predict that buckling strengths can be dramatically eroded by small unavoidable manufacturing imperfections: this is confirmed by tests on stiffened panels of box-girder bridges. A related analysis yields a spectacular picture of the hyperbolic-umbilic catastrophe.

Two parallel and complementary contributions to our understanding of evolutionary instabilities have recently been presented. The first is the widely acclaimed qualitative catastrophe theory of René Thom¹ which has been applied most fruitfully by Zeeman and others to problems in sociology, economics and theoretical biology².

The second is the quantitative general branching theory of Thompson and Hunt³ which has had a major impact on our approach to the instability of elastic solids. This is essentially a prototype bifurcation theory for slowly evolving systems in the spirit of Poincaré, and has wide applications across the mathe-

matical sciences in such areas as cosmology, hydrodynamics and crystallography.

The two theories have been developed quite independently, but are remarkably similar in form and content, and Hunt and I have just completed a preliminary correlation between them⁴. Cross fertilisation between the two disciplines promises to be most rewarding, and catastrophe theorists may be interested to find in ref. 3 well developed perturbation schemes for the quantitative analysis of bifurcations and catastrophes, together with a wealth of catastrophe machines akin to that of Zeeman⁵.

General theory

In their simplest form, the two theories relate to any system governed by a potential function $V(Q_i, \Lambda^j)$ where Q_i is a set of n state variables or generalised coordinates, and Λ^j is a set of k externally controlled parameters. Stationary values of V with respect to the Q_i are supposed to be necessary and sufficient for equilibrium, while minimum values are supposed to be

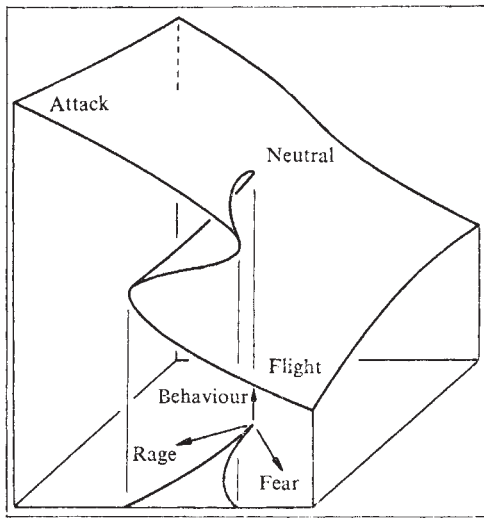


Fig. 1 Zeeman's illustration of the cusp catastrophe in the response of a dog subjected simultaneously to fear and rage.

necessary and sufficient for stability. The n equilibrium equations $\partial V/\partial Q_i = 0$ thus define surfaces⁶ in the $k+n$ dimensional $(\Lambda-Q)$ space, while the vanishing of the stability determinant $|\partial^2 V/\partial Q_i \partial Q_j|$ identifies critical equilibrium states at which a loss of stability can be expected.

The locus of critical states projected into the k -dimensional control space gives a catastrophe boundary which in elastic stability usually corresponds to an imperfection-sensitivity curve on a plot of the failure load against the imperfection magnitude. Usually Λ^j is finally assumed to be slowly varying, and attention is focused on the steady evolution of an initially stable equilibrium solution which can be destroyed by a sudden dynamic snap from one of the critical equilibrium states.

The two theories delineate the forms of instability that can occur by classifying the singularities that can arise in the catastrophe boundary, and a powerful result of topology allows Thom to identify seven "ordinary catastrophes". These are the only topologically stable, and therefore the only naturally occurring, forms that can arise in a control space of up to four dimensions ($k \leq 4$) no matter how many state variables Q_i there are in the system.

This result has significance in developmental biology where the external control parameters of a cell might be its spatial coordinates (x, y, z) and the time t , the former influencing the cell through the spatially varying chemical environment. Our conceptual catastrophe boundary now becomes a four-dimensional spatio-temporal boundary of behaviour⁷.

Two applications

In the past, bifurcation and catastrophe theorists have tended to view their identical problems in rather different ways, and I can best illustrate this by means of two illustrative examples.

An attractive illustration of qualitative catastrophe theory in an inexact science is Zeeman's demonstration of the cusp phenomenon in the psychological response of a dog or a man subjected simultaneously to feelings of fear and rage. Here we must think of the intensities of fear and rage as being two controlled variables Λ^j under which we observe the behaviour of the dog as a single coordinate Q . We thus expect to have a response surface which Zeeman⁸ argues must have the form shown in Fig. 1.

Under low stimulus we see that the behaviour can vary continuously from flight to attack, but under high stimulus the dog's response varies discontinuously with jumps in behaviour at the fold-lines which project into a cusp in the plan view. Clearly it is imperative for the animal that under intense pressure it should not respond in a neutral manner, even if

fear and rage are equally balanced, and correspondingly the inverted fold of the surface represents unrealisable unstable states.

By contrast, a quantitative application⁹ of the general bifurcation theory is sketched schematically in Fig. 2. Here a square sheet of crystal is loaded by a direct force or stress σ_{11} and deforms accordingly into a rectangle with a direct elongation or strain ϵ_{11} . At a certain load, however, a point of bifurcation C is encountered at which a symmetry-destroying shearing deformation ϵ_{12} develops and the crystal deforms unexpectedly into a parallelogram. At this bifurcation load the directly stressed crystal would fail explosively in a homogeneous shearing mode. A small constant shearing stress σ_{12} would clearly induce some shearing strain ϵ_{12} even before the bifurcation, so such a stress would act as an "imperfection" rounding off the bifurcation as shown.

In this example the stresses σ_{11} and σ_{12} are to be thought of as two control parameters Λ^j whereas the strains ϵ_{11} and ϵ_{12} are to be identified as two state variables Q_i . The first picture shows equilibrium paths in a three-dimensional graph, a significant projection of which is shown underneath on the left hand side. The load carrying capacity as measured by the maximum direct stress σ_{11}^M is severely reduced by the application of even a small σ_{12} , and the locus of critical points is finally projected into the control space to give a cusp-shaped failure stress locus of σ_{11}^M as a function of σ_{12}^M .

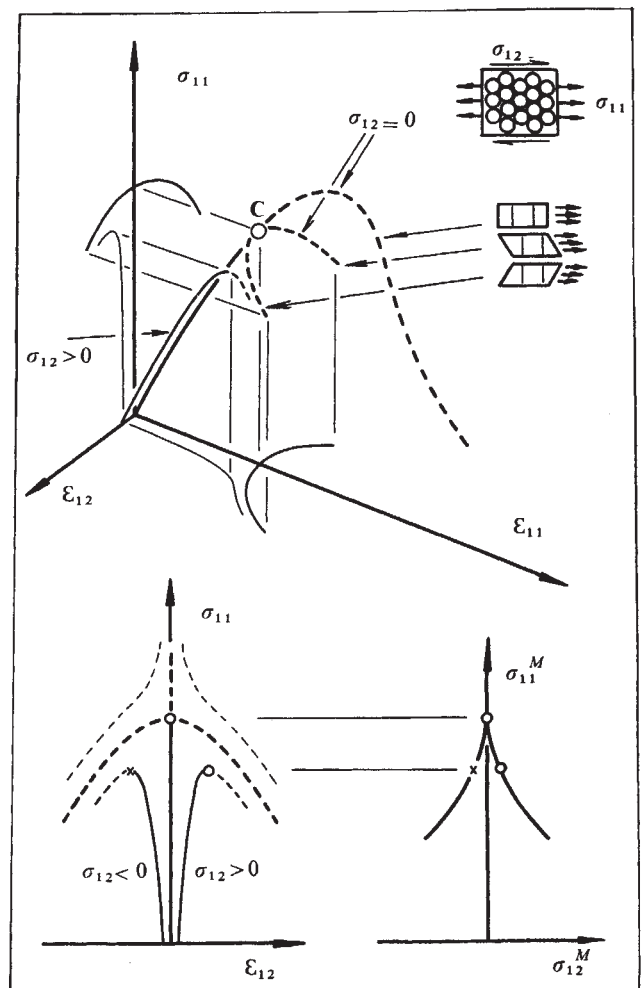


Fig. 2 The demonstration by Thompson and Shorrock of a bifurcation in the response of a mechanically stressed atomic lattice. A close-packed crystal with Lennard-Jones interatomic potentials is considered and the primary path is shown to lose its stability in a symmetry-destroying unstable-symmetric point of bifurcation at C . This gives rise to a cusp on the failure stress locus. In an extension of this work we have shown that the crystal exhibits a hyperbolic-umbilic catastrophe in the three dimensional failure space of the planar stresses σ_{11} , σ_{12} and σ_{33} .

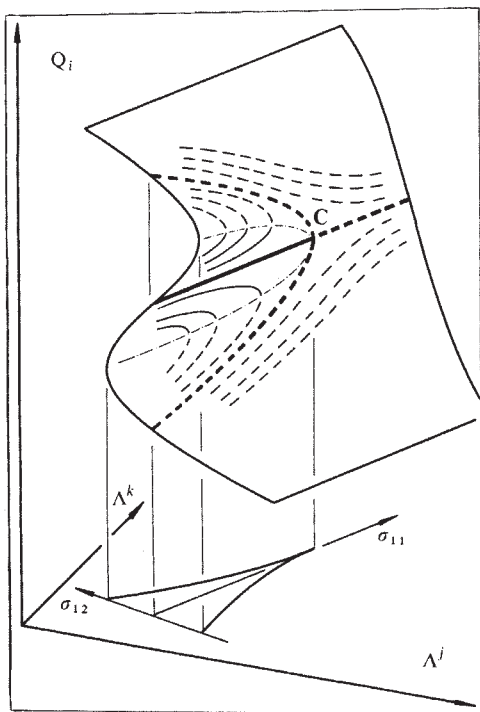


Fig. 3 Paths on an equilibrium surface demonstrating the equivalence between the unstable-symmetric point of bifurcation and the cusp catastrophe. One distinction between the examples of Fig. 1 and Fig. 2 is that in the former the bulk of the surface is stable and the inverted fold is unstable while in the latter the situation is exactly reversed. My stable- and unstable-symmetric points of bifurcation are each classified by Thom as a cusp catastrophe.

Figure 1, with its emphasis on the whole equilibrium surface, is an example of Thom's Riemann-Hugoniot cusp catastrophe and Fig. 2, with its emphasis on equilibrium paths, represents my unstable-symmetric point of bifurcation. But in fact these are one and the same phenomenon, as is made clear in Fig. 3 in which bifurcating paths are generated simply by slicing the folded equilibrium surface at constant σ_{12} .

This cusp, or distinct symmetrical point of bifurcation, which generates a two-thirds power law cusp in Λ space, is by far the most common branching phenomenon and can be found throughout the mathematical sciences, one significant example arising in the instability of rotating stellar masses^{10,11}.

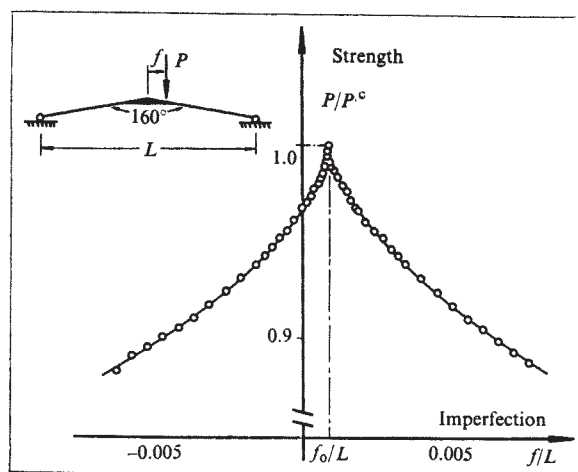


Fig. 4 Test by Roorda on a model arch, showing a deterioration of strength with the controlled asymmetry.

Elastic arch

This ubiquitous cusp was first studied experimentally by Roorda at University College, London, and one of his many tests is summarised in Fig. 4. Here a shallow steel arch pinned to rigid abutments is loaded, nominally at its crown, by a load P which is progressively offset by a small distance f to simulate an asymmetric manufacturing imperfection. The behaviour of the arch is elastic throughout, and failure is associated with the dynamic snapping of the arch through asymmetric configurations into an inverted state.

In this example the load P and the offset f are to be regarded as two controlled parameters while the in-plane rotation of the crown, θ , can be viewed as a single generalised coordinate measuring the asymmetric deformation. The equilibrium paths

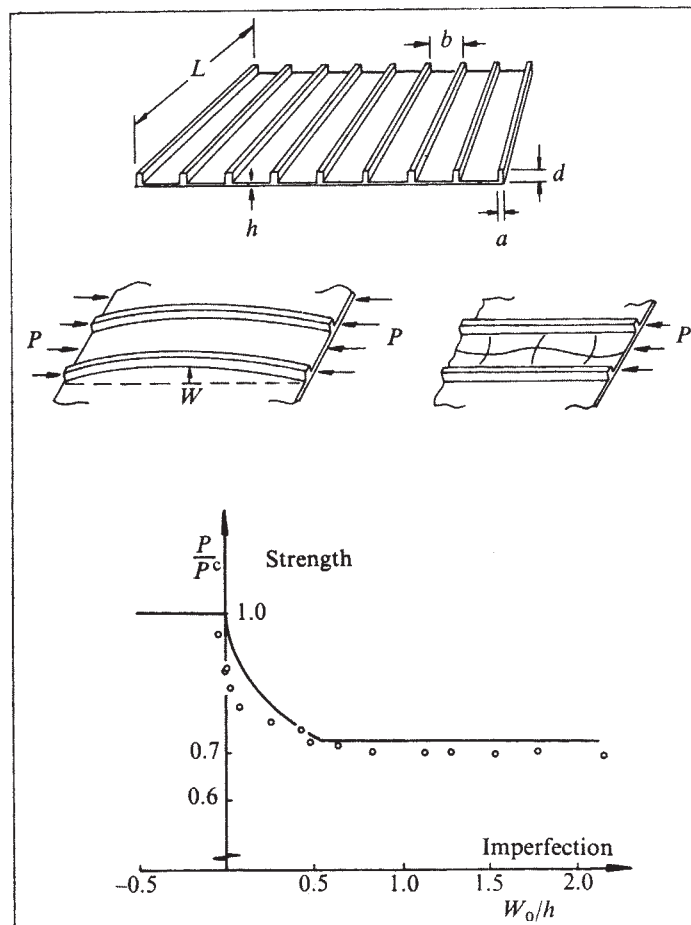


Fig. 5 The sensitivity of the strength of a stiffened panel to manufacturing imperfections W_0 in the overall form. The model panel tested was of epoxy plastic with dimensions $h=0.75$ mm, $b=57.5$ mm, $a=4.9$ mm and $d=10$ mm. The graph corresponds to a section through the hyperbolic-umbilic catastrophe of Fig. 6. This test is one of a continuing series conducted by A. C. Walker.

of the family of arches generated by varying f have the general form of the unstable-symmetric point of bifurcation shown in the lower graphs of Fig. 2 with P replacing σ_{11} , f replacing σ_{12} and θ replacing ε_{12} .

The right-hand-side cusp is of particular interest and now represents an imperfection-sensitivity curve on a plot of the failure load P as a function of the simulated imperfection magnitude f . The curve determined experimentally is shown in Fig. 4. This follows the predicted two-thirds power law, and shows a dramatic deterioration of strength away from the optimum 'design' at $f=f_0$ at which the critical offset f_0 is just cancelling out the unavoidable manufacturing errors in the test specimen (see also ref. 3).

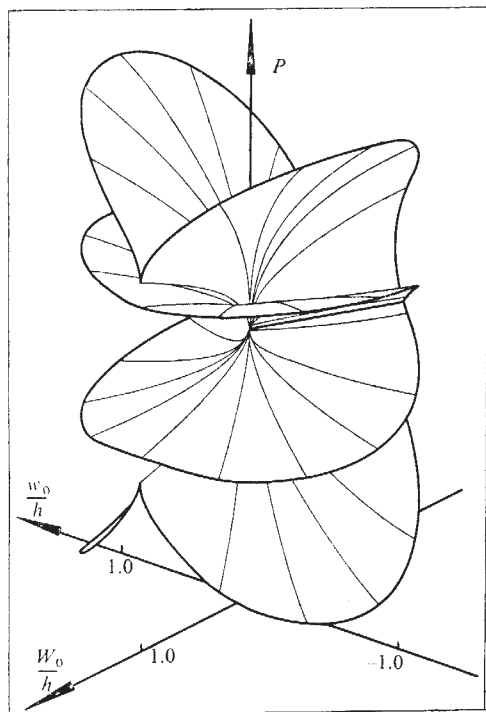


Fig. 6 The hyperbolic-umbilic catastrophe determined by Hunt (unpublished) in the theoretical response of a stiffened elastic panel. The lower region of the surface gives the panel strength as a function of the overall and local manufacturing imperfections⁴.

Highly optimised structures

I have just presented an example of an optimum design which is extremely sensitive to unavoidable manufacturing imperfections, and it is my thesis^{3,12} that as higher and higher degrees of optimisation are sought, so the severity both of the failure and of the associated imperfection-sensitivity will rise. Indeed this is likely to be a feature of any highly optimised system, whether in the field of engineering structures or not.

As an illustration of this I consider the elastic buckling of an optimised stiffened panel similar to those employed in box-girder bridges. The model panel of Fig. 5 is compressed by an axial load P , parallel to the stiffeners, under which the specimen may deflect like a simple column in an overall mode with amplitude W as shown on the left, or may alternatively ripple between the stiffeners in a local mode with amplitude w as indicated on the right.

The much used, but potentially dangerous concept of simultaneous mode design^{3,12} states that the structure will be optimal when these two failure modes can develop simultaneously, and I have chosen the length of the panel so that this condition is closely realised. A simulated initial deflection of amplitude W_0 in the overall mode represents a manufacturing imperfection, and we¹³ have obtained the imperfection-sensitivity curve shown, failure being characterised by a sudden dynamic snap in the range of material elasticity. A dangerous erosion of strength with positive imperfection amplitude is indicated, and the experimental points are seen to be in quite good agreement with the curves of a simplified *ad hoc* theoretical treatment.

This treatment and more details of the tests are to be reported elsewhere¹³. They show that highly optimised plate assemblages must be expected to have the severe imperfection-sensitivity characteristics previously associated only with the notorious buckling behaviour of thin shells, a conclusion that will undoubtedly carry over into the plastic range.

Hyperbolic-umbilic catastrophe

A similar stiffened elastic panel containing additionally a local imperfection of amplitude w_0 has been analysed more rigorously by V. Tvergaard, and his asymptotic equations corresponding to our homeoclinic point of bifurcation have been solved in detail by G. W. Hunt (unpublished) to give the complete imperfection-sensitivity surface of Fig. 6, the lower region of which gives the panel strength as a highly-peaked function of the two imperfection amplitudes W_0 and w_0 . This dramatic computed picture has been identified by D. Chillingworth of Southampton University as a hyperbolic-umbilic catastrophe, and this seems to be the first known practical example of this predicted form.

Received December 13, 1974.

- ¹ Thom, R., *Stabilité Structurale et Morphogénèse* (Benjamin, New York, 1972); *Topology*, 8, 313-335 (1969).
- ² Zeeman, E. C., *Applications of catastrophe theory*, Tokyo International Conference on Manifolds (in the press).
- ³ Thompson, J. M. T., and Hunt, G. W., *A General Theory of Elastic Stability* (Wiley, London, 1973).
- ⁴ Thompson, J. M. T., and Hunt, G. W., *J. appl. Math. Phys.* (in the press).
- ⁵ Zeeman, E. C., *A catastrophe machine*, in "Towards a Theoretical Biology", 4 (edit. by Waddington, C. H.) (Edinburgh University Press, Edinburgh, 1972).
- ⁶ Huseyin, K., *Nonlinear Theory of Elastic Stability* (Noordhoff, Leyden, 1974).
- ⁷ Zeeman, E. C., in *Lectures on Mathematics in the Life Sciences*, 7 (Am. Math. Soc., Providence, in the press).
- ⁸ Zeeman, E. C., *Times Lit. Suppl.*, December 10, 1556-7 (1971).
- ⁹ Thompson, J. M. T., and Shorrock, P. A., *J. Mech. Phys. Solids*, 23, 21-37 (1975).
- ¹⁰ Lytleton, R. A., *The Stability of Rotating Liquid Masses*, (Cambridge University Press, Cambridge, 1953).
- ¹¹ Ledoux, P., in *Handbuch der Physik*, II (edit. by Flugge, S.), (Springer, Berlin, 1958).
- ¹² Thompson, J. M. T., and Hunt, G. W., *Engineering Optimization*, 1, 99-110 (1974).
- ¹³ Thompson, J. M. T., Tulk, J. D., and Walker, A. C., in *Proc. Harvard Symp. Buckling of Structures* (Springer, in the press).

Evolution of Precambrian crust from strontium isotopic evidence

S. Moorbath

Department of Geology and Mineralogy, Oxford University, Oxford, UK

The strontium isotope evidence places constraints on the evolution of Precambrian crust. Contrary to popular belief, sialic crust cannot, it seems, be "reworked" on a grand scale.

MANY geologists believe intuitively that Archaean and post-Archaean gneisses, the ultimate origin of which is not recognisable in the field, are somehow derived from "much older" sialic (that is, continental igneous, sedimentary or metamorphic) crust. A particularly popular hypothesis is that continental,

sialic crust can somehow be "reworked" repeatedly on a grand scale. (The term reworked is here used in the sense of partial or complete melting leading to mobilisation and reconstitution as an essentially new rock and not in the frequently used, but incorrect, sense of isochemical recrystallisation and/or deformation.) These views are not compatible with the results of some recently published Sr isotopic data on Archaean and Proterozoic gneisses, and greenstone belt volcanic rocks with associated later granodioritic or tonalitic plutons.

The views presented below are an extension of those of

Spooner and Fairbairn¹, who reported low, if somewhat imprecisely determined, initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for several suites of Precambrian pyroxene granulite gneisses. In particular, they favoured an igneous origin for such rocks, involving transfer into the crust of a mantle-derived magma of approximately andesitic composition. Spooner and Fairbairn suggest that the part that reaches the upper crust will crystallise as normal extrusive and intrusive rocks, but any part that is trapped in the lower crust at about 10^5 KPa will cool slowly to a minimum of about 500°C and crystallise directly into plagioclase-pyroxene assemblages, characteristic of the granulite facies. In this connection, igneous rocks with magmatic features characteristic of fractional crystallisation but with typical granulite facies mineralogy are well known from such areas as Lofoten-Vesteraalen, north Norway, where large quantities of mangeritic and charnockitic magmas were intruded at depths of 20–25 km and at $900\text{--}1,000^\circ\text{C}$ (ref. 2).

Geochemistry and isotope data

The geochemistry of major elements of many pyroxene granulite and amphibolite facies gneisses (see, for example, ref. 3) suggests strong affinity with igneous rocks of calc-alkaline character, in spite of the characteristic depletion in certain incompatible trace elements such as U, which shows up so particularly well in the present unradiogenic Pb isotope ratios in many high-grade gneisses^{4,5}. This depletion occurred within the uncertainty of the measured whole rock Pb/Pb age and has been interpreted as an essentially metamorphic phenomenon^{4,5}. The highest grade gneisses sometimes also show strong synmetamorphic Rb depletion, leading to present unradiogenic Sr isotope ratios^{4,6}.

Table 1 is a summary of recently reported Rb–Sr whole rock ages and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of some major Precambrian gneiss complexes. Also shown are measurements on basaltic-to-andesitic greenstone belt volcanics and associated (either cross-cutting or presumed later) calc-alkaline plutons from two areas. Almost all initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are in close agreement in the general range $0.700\text{--}0.702$, and fall rather close to the value presumed to be characteristic of the upper mantle for the corresponding age⁷.

Figure 1 shows averaged $^{87}\text{Sr}/^{86}\text{Sr}$ growth lines, calculated from the mean of the measured Rb/Sr values for each individual rock unit in the publications cited in Table 1. The slopes of the lines are proportional to the Rb/Sr ratios, which are mostly within the range $0.2\text{--}0.4$ for the gneisses and granites listed in Table 1, although each rock unit exhibits considerable dispersion in Rb–Sr values. The data for Greenland, Scotland and Rhodesia are plotted in relation to a hypothetically linear upper mantle growth line extending from 0.699 to 0.703 in $4,600$ Myr, corresponding to a Rb/Sr ratio of about 0.02 . (It is not yet known for certain whether this line is strictly linear, or slightly convex upwards implying nonlinear evolution of upper mantle Sr⁷.) The positions of all initial ratios fall almost on, or slightly above, the linear upper mantle growth line, and demonstrate beyond any reasonable doubt that later rock units cannot have been derived by the reworking of earlier ones of the type exposed in any given region. The same conclusion applies to the North American data, which are not plotted because of the large grouping of ages at around $2,700$ Myr and the relative uncertainty in the values for the Minnesota River Valley gneiss.

Implications for evolutionary models

The measured ages and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for unaltered greenstone belt volcanics and later plutons almost certainly relate to the time of crystallisation. For a gneiss complex, they could relate to the time of regional metamorphism. In that case, linear extrapolation of any given $^{87}\text{Sr}/^{86}\text{Sr}$ growth line back to the linear upper mantle growth line yields an upper age limit for the precursors of the gneiss complex. In most cases this does not exceed about 100 Myr, and in some cases it is less than 50 Myr. Since high-grade metamorphism may cause a decrease in Rb/Sr ratios, the first part of the $^{87}\text{Sr}/^{86}\text{Sr}$ growth line could have been significantly steeper, so that the age difference between formation of the precursors and their metamorphism would be correspondingly reduced. A nonlinear upper mantle growth line (convex upwards) would, of course, have much the same effect.

Several major Archaean and Proterozoic gneiss complexes, as well as greenstone belt volcanics and associated later plutons, are predominantly juvenile additions to the continental crust at, or close to, the measured age, implying continental growth on a major scale during the Precambrian (Table 1). Furthermore, the

Table 1 Rb–Sr whole rock ages and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of Precambrian rock units

Rock unit and area	Age (Myr)*	Initial $^{87}\text{Sr}/^{86}\text{Sr}$
Greenland and Scotland		
Amîtsoq gneiss, Godthaab and Isua areas, West Greenland ²³ .	$3,750 \pm 50$	0.7010 ± 0.0005
Nûk Gneiss, Godthaab, West Greenland ²⁴ .	$3,040 \pm 50$	0.7026 ± 0.0004
"Nûk" Gneiss, Sermilik, West Greenland†.	$3,110 \pm 80$	0.7010 ± 0.0010
Ketilidian granite gneiss, South-west Greenland ²⁵ .	$1,890 \pm 90$	0.7022 ± 0.0010
	$1,780 \pm 20$	0.7032 ± 0.0005
Grey gneiss, Outer Hebrides, Scotland ²⁶ .	$2,690 \pm 140$	0.7014 ± 0.0007
Rhodesia		
Basement granite gneiss ^{27,28} .	$3,600 \pm 200$	0.701 ± 0.001
Gwenoro gneiss ²⁸ .	$2,780 \pm 30$	0.7011 ± 0.0001
Maliyami formation (Main greenstone belt) ²⁸ .	$2,720 \pm 70$	0.7010 ± 0.0002
Sesombi tonalite pluton ²⁸ .	$2,690 \pm 70$	0.7008 ± 0.0004
North America		
a Greenstone belt volcanics		
Michipicoten, Ontario ²⁹ .	$2,600 - 2,700$	0.7018 ± 0.0002
Coutchiching and Keewatin, Minnesota and Ontario ^{30,31} .	$2,600 - 2,700$	0.7010 ± 0.0004
Yellowknife, NWT ¹² .	$2,625 \pm 160$	0.7022 ± 0.0023
b Gneisses and granites		
Minnesota River Valley Gneiss ³²	approx. 3800 (?)	approx. 0.700 (?)
Northern Light, Saganaga, Icarus, Grants Range, Algoman, Vermilion, Minnesota–Ontario border ^{30,33,34} .	$2,600 - 2,700$	0.7010 ± 0.0005
Yellowknife, NWT ¹² .	$2,640 \pm 40$	0.7011 ± 0.0008
Chibougamou, Quebec ³⁵ .	$2,740 \pm 230$	0.7009 ± 0.0005
	$2,680 \pm 80$	0.7005 ± 0.0002
Wind River Range, Wyoming ³⁶ .	$2,630 \pm 20$	0.702 ± 0.001
Twilight Gneiss, Colorado ³⁷ .	$1,805 \pm 35$	0.7015 ± 0.0004

* ^{87}Rb decay constant $1.39 \times 10^{-11} \text{ yr}^{-1}$.

†R. J. Pankhurst and S. M., unpublished.

interval between the time of extraction of juvenile igneous material from the upper mantle (and/or subducted oceanic lithosphere) and the time of production of the regional metamorphic characteristics of the derived gneiss complex may be less than 50–100 Myr, which falls within the analytical uncertainty of most age measurements of Archaean and Proterozoic rocks. It is possible that during such an "accretion" event, major amounts of juvenile igneous material broadly equivalent in composition to the island arc tholeiite series and, in particular, to the calc-alkaline series, were produced at depth in an ancient proto-island arc regime^{8–11}. Some of this material came directly to the surface of the pre-existing continental or oceanic crust where it gave rise to the volcanic assemblage characteristic of greenstone belts. The remainder crystallised at depth as the hypabyssal and plutonic equivalents of the high level rocks. The

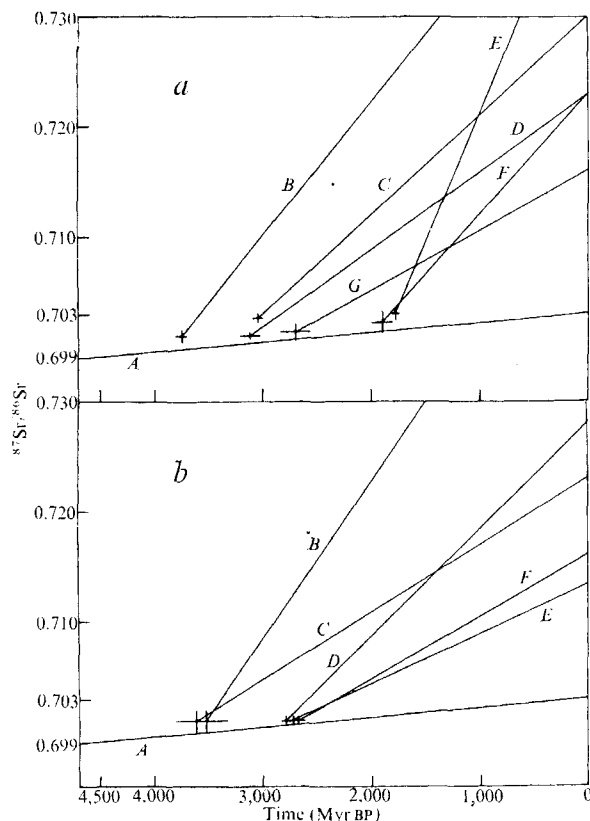


Fig. 1 Average $^{87}\text{Sr}/^{86}\text{Sr}$ growth lines for: *a*, Greenland and Scotland. *A*, upper mantle growth line; *B*, Amitsoq gneiss, Godthaab and Isua areas²³; *C*, Nûk gneiss, Godthaab area²⁴; *D*, "Nûk" gneiss, Sermilik area (Oxford unpublished data); *E, F*, Ketilidian granite gneisses, south-west Greenland²⁵; *G*, Grey gneiss, Outer Hebrides, Scotland²⁶. *b*, Rhodesia. *A*, upper mantle growth line; *B*, Mushandike granite gneiss²⁷; *C*, Mashaba granite gneiss²⁸; *D*, Gwonor migmatitic gneiss²⁸; *E*, Maliyami formation greenstone belt²⁸; *F*, Sesombi tonalite pluton (cross-cutting the Maliyami formation)²⁸.

volcanic association is probably the early extrusive expression of the subcrustal processes which later led to the intrusion of calc-alkaline plutons into the deforming volcanic-sedimentary pile, as already suggested for the Yellowknife Province by Green and Baadsgaard¹². At depth, the plutonic material slowly crystallises with igneous or metamorphic mineral assemblages, simultaneously undergoing geochemical and petrological differentiation to give a compositionally layered lower crust^{13–17}, in which the major and minor components of granite (*sensu lato*) migrate upwards to form rocks of the calc-alkaline plutonic association, leaving behind a thick residue of depleted granulite facies rocks at greater depth. The resulting crustal layering is gradational and highly complex in detail. Under these plutonic conditions the

conventional distinction between what is "igneous" and what is "metamorphic" is no longer clearcut. Furthermore, intermediate zones of variably depleted calc-alkaline amphibolite facies gneisses must also be quantitatively important. Most of the gneisses in Table 1 are actually of this type.

In some cases, upward migrating volcanic and plutonic materials penetrate much older continental, sialic crust (as in Rhodesia) which could have formed in much the same way during an earlier continental accretion episode. But this older sialic crust, where present, may contribute little or no "reworked" material (Fig. 1), although it may well undergo renewed metamorphism and tectonism (as in the Godthaab area of West Greenland), as well as providing xenoliths and enclaves of many different types in the younger volcanic and plutonic rocks. But it seems, in principle, unlikely that relatively low density, continental sialic crust can be buried or subducted sufficiently deeply for really large scale reworking of basement gneisses of the type postulated by many geologists (see, for example, refs 18 and 19). True reworking of older sialic crust may, of course, happen on a limited scale above ancient and modern subduction zones, and it is quite possible that in certain tectonic regimes such as in orogenic belts formed in sites of continental collision with resulting major crustal thickening, sialic crust can be truly reworked on a moderate scale²⁰. Localised partial melting of ancient, sialic crust may also occur in the zone of heating above large bodies of basic igneous magma introduced into high crustal levels, as for example in the Tertiary igneous province of north-west Scotland²¹. All of these complicating factors which involve partial melting of, and assimilation with, ancient sialic crust are clearly exhibited by the complexities of initial Sr isotopic ratios in many relatively high level granites and related rocks of mainly Phanerozoic age⁷.

The mechanisms of continental growth and crustal evolution implied by plate tectonics may well be adequate to explain the evolution of major Precambrian crustal units, although there is no final proof. At any rate, they are certainly compatible with the Sr isotopic evidence. The sequence of events comprises: plate formation—plate separation (ocean floor spreading)—plate convergence—subduction—partial melting of basic lithosphere and/or upper mantle—extraction and emplacement of basaltic and calc-alkaline volcanic and plutonic rock types—accretion of continental crust—geochemical and petrological differentiation of new continental crust. The Sr isotopic evidence suggests that this sequence can be relegated for any given cratonic suite, which exhibits close grouping of rock-forming ages and low initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, into a continuous or semi-continuous "super-event" not exceeding 50–100 Myr in duration. This value is of the same order as found at present between the most recently formed complementary ocean ridges, island arcs and continental margins.

The major conclusions drawn from the Sr isotopic evidence are, of course, unaffected by the particular model chosen for continental growth, whether plate-tectonic or otherwise. But for the examples cited here, the process of repeated reworking of significantly older sialic crust with anything like normal crustal Rb/Sr ratios must be rejected.

Received January 21, 1975.

- 1 Spooner, C. M., and Fairbairn, H. W., *J. geophys. Res.*, **75**, 6706 (1970).
- 2 Griffin, W. L., Heier, K. S., Taylor, P. N., and Weigand, P. W., *Norges Geol. Undersøk.* (in the press).
- 3 Sheraton, J. W., Skinner, A. C., and Tarney, J., in *The Early Precambrian of Scotland and Related Rocks of Greenland* (edit. by Park, R. G., and Tarney, J.) (University of Keele, 1973).
- 4 Moorbath, S., Welke, H., and Gale, N. H., *Earth planet. Sci. Lett.*, **6**, 245 (1969).
- 5 Black, L. P., Gale, N. H., Moorbath, S., Pankhurst, R. J., and McGregor, V. R., *Earth planet. Sci. Lett.*, **12**, 245 (1971).
- 6 Evans, C. R., *Nature*, **207**, 54 (1965).
- 7 Faure, G., and Powell, J. L., *Strontium Isotope Geology* (Springer, 1972).
- 8 Ringwood, A. E., *J. geol. Soc. Lond.*, **130**, 183 (1974).
- 9 Brown, G. C., *Nature phys. Sci.*, **241**, 26 (1973).
- 10 Barker, F., and Peterman, S. E., *Precambrian Res.*, **1**, 1 (1974).
- 11 White, A. J. R., Jakes, P., and Christie, D. M., *Spec. Publ. No. 3*, 47 (Geol. Soc. Austral., 1971).
- 12 Green, D. C., and Baadsgaard, H., *J. Petrol.*, **12**, 177 (1971).
- 13 Fyfe, W. S., *Geol. J. Spec. Issue No. 2*, 201 (1970).
- 14 Fyfe, W. S., *Tectonophysics*, **17**, 273 (1973).
- 15 Brown, G. C., and Fyfe, W. S., *Contr. Mineral. Petrol.*, **28**, 310 (1970).
- 16 Heier, K. S., *Fortschr. Miner.*, **50**, 174 (1973).

- ¹⁷ Heier, K. S., *Phil. Trans. R. Soc.*, **A273**, 429 (1973).
¹⁸ Bridgwater, D., Watson, J., and Windley, B. F., *Phil. Trans. R. Soc.*, **A273**, 493 (1973).
¹⁹ Bridgwater, D., McGregor, V. R., and Myers, J. S., *Precambrian Res.*, **1**, 179 (1974).
²⁰ Dewey, J. F., and Burke, K. C. A., *J. Geol.*, **81**, 683 (1973).
²¹ Moorbath, S., and Bell, J. D., *J. Petrol.*, **6**, 37 (1965).
²² York, D., and Farquhar, R. M., *The Earth's Age and Geochronology* (Pergamon, 1972).
²³ Moorbath, S., O'Nions, R. K., Pankhurst, R. J., Gale, N. H., and McGregor, V. R., *Nature phys. Sci.*, **240**, 78 (1972).
²⁴ Pankhurst, R. J., Moorbath, S., and McGregor, V. R., *Nature phys. Sci.*, **243**, 24 (1973).
²⁵ Van Breemen, O., Aftalion, M., and Allaart, J. H., *Geol. Soc. Am. Bull.*, **85**, 403 (1974).
²⁶ Moorbath, S., Powell, J. L., and Taylor, P. N., *J. geol. Soc. Lond.* (in the press).
²⁷ Hickman, M., *Nature*, **251**, 295 (1974).
²⁸ Hawkesworth, C. J., Moorbath, S., O'Nions, R. K., and Wilson, J. F., *Earth planet. Sci. Lett.* (in the press).
²⁹ Hart, S. R. and Brooks, C., *Yb. Carnegie Instn Wash.*, **68**, 426 (1968–1969).
³⁰ Peterman, Z. E., Goldich, S. S., Hedge, C. E., and Yardley, D. H., *Geol. Soc. Am. Mem.*, **135**, 193 (1972).
³¹ Hart, S. R., and Davis, G. L., *Geol. Soc. Am. Bull.*, **80**, 595 (1969).
³² Goldich, S. S., and Hedge, C. E., *Nature*, **252**, 467 (1974).
³³ Hanson, G. N., Goldich, S. S., Arth, J. G., and Yardley, D. H., *Can. J. Earth Sci.*, **8**, 1110 (1971).
³⁴ Prince, L. A., and Hanson, G. N., *Geol. Soc. Am. Mem.*, **135**, 217 (1972).
³⁵ Jones, L. M., Walker, R. L., and Allard, G. O., *Can. J. Earth Sci.*, **11**, 1550 (1974).
³⁶ Naylor, R. S., Steiger, R. H., and Wasserburg, G. J., *Geochim. cosmochim. Acta*, **34**, 1133 (1970).
³⁷ Barker, F., Peterman, Z. E., and Hildreth, R. A., *Contr. Mineral. Petrol.*, **23**, 271 (1969).

letters to nature

Energy spectrum of diffuse component of cosmic soft γ rays

In this paper we present new measurements on the diffuse component of cosmic soft gamma rays in the energy range 0.1–4 MeV obtained with a balloon-borne telescope. These measurements show that the energy spectrum gradually steepens from $E^{-2.3}$ to $E^{-2.8}$ as the energy increases from 100 to 500 keV and becomes less steep thereafter, consistent with the presence of a hump in the MeV region.

Previous observations^{1–5} of the diffuse gamma ray spectrum obtained both at balloon altitudes and with detectors on board spacecrafts are subject to criticism. In the spacecraft observations^{6,7}, the counting rate in the detectors resulting from radioactivity induced by cosmic ray interactions is comparable to that of the cosmic component of gamma rays at energies above 1 MeV and the corrections for this effect are not free from ambiguity. In balloon observations the procedure for deducing the background contribution from secondary cosmic rays by linearly extrapolating the growth curve is not justified for omnidirectional detectors^{8–10}. These difficulties can be circumvented by employing the "active shutter method" described previously¹¹.

This experiment is an improved version of the scheme with which a well defined directional response for the gamma ray detection has been achieved previously. The central NaI (TI) counter of 7.5 cm diameter and 7.5 cm thickness is surrounded by a cylindrical NaI (TI) counter of 10 cm wall thickness and 30 cm height. The bottom hole is covered by a CsI (TI) crystal, 7.5 cm in diameter and 7.5 cm thick. A shutter counter made of NaI (TI) of 7.5 cm diameter opens and closes the entrance aperture at the top for alternate periods of one minute. By taking the difference between the counting rates of the open and closed modes, the background caused by the radiation outside the entrance aperture is eliminated. The angular response of the detector for this difference is nearly triangular with FWHM of about 40°, as determined by using gamma ray sources ⁵⁷Co (122 keV), ¹³⁷Cs (660 keV), ⁶⁰Co (1.7, 1.33 MeV) and ²⁴Na (1.37, 2.75 MeV). The geometrical factor at 1.2 MeV is 23 cm² sr. The detection threshold of the guard counters and the shutter counter are set at 15 keV. The response of the detector to neutrons has been measured using an Am–Be source; the counting rate of the telescope at the ceiling has a negligible contribution from atmospheric neutrons.

The gamma ray detector was flown from Hyderabad, India on April 13, 1974. The balloon stayed at the ceiling altitude of 4,300 Pa (4.3 mbar) for about two hours and then gradually

descended to 6,800 Pa over the next three hours. The pulse heights measured in the central counter were analysed into 128 channels and telemetered event by event along with the indication of coincidence with the shutter and/or the guard counters. The counting rates of the guard counters were also monitored. In-flight calibrations of the central and shutter counters revealed showed that the functioning of the apparatus was stable throughout the flight.

The pulse height distributions observed at the ceiling altitude are shown in Fig. 1 for the open and the closed modes of operation of the telescope. Their difference, also shown in the same figure, represents the pulse height distribution for the 'forward gamma rays'. To determine the gamma ray intensity at the top of the atmosphere the following functional form for

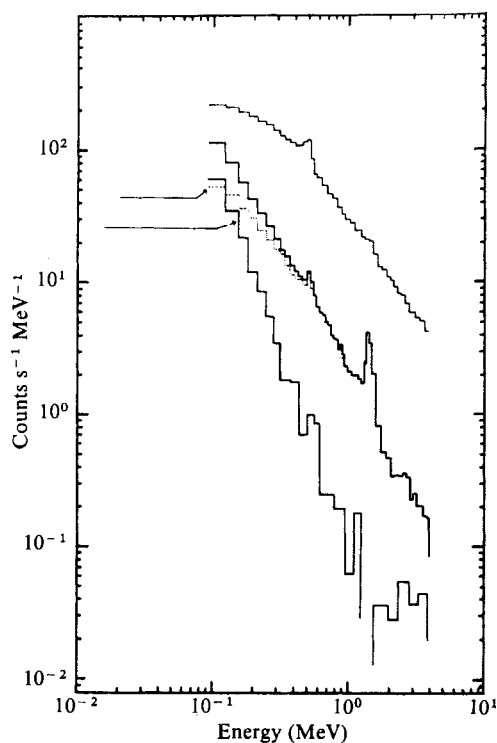


Fig. 1 Observed pulse height distributions of the central counter at the ceiling altitude. a, Anti-off, open; b, anti-on, open; c, closed; d, difference (open-closed). "Anti-off" indicates guard counter pulses disregarded.

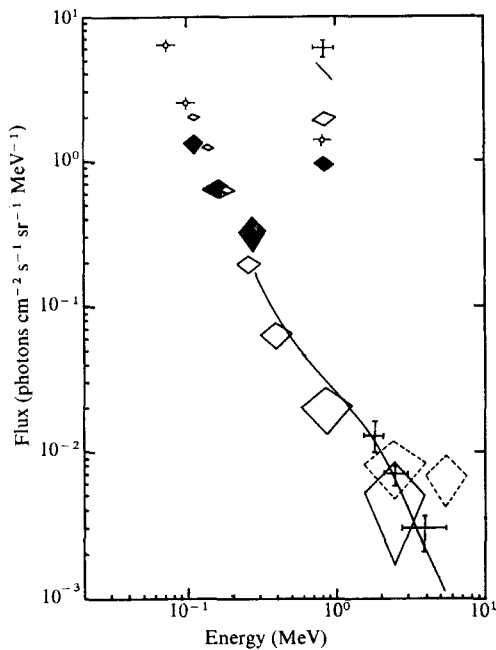


Fig. 2 Energy spectrum of soft gamma rays. The present result is indicated by open diamonds. Dashed diamonds represent the fluxes not corrected for the electron contribution. $\blacklozenge$ and $\circ$, Our previous balloon observations; +, Schönfelder and Lichti (unpublished; solid line, ref. 5.)

the growth curve was assumed for altitudes higher than 50 g cm^{-2} :

$$C(z) = I_0 F(z) + I_b z$$

where $C(z)$ is the counting rate at an altitude z (in g cm^{-2}), I_0 is the intensity of primary gamma rays, $F(z)$ is the attenuation function of primary gamma rays in air and I_b is the constant. The last term is the intensity of the atmospheric component, and its assumed linear z -dependence for the narrow aperture telescope used in the present experiment is supported by Monte Carlo calculations⁸⁻¹⁰. $F(z)$ is taken from a Monte Carlo calculation by Horstman and Horstman-Moretti¹².

The energy loss spectrum has been transformed to photon spectrum by the use of response functions obtained for different energies using radio isotopes. A comment is necessary for the data above 1 MeV. Since the entrance aperture is not protected against charged particles, re-entrant albedo electrons are suspected to give an appreciable contribution in this energy region. This has been estimated using the observed electron spectrum⁶. The spectrum of the gamma rays thus derived with the utmost correction of the electrons is shown by open diamonds, whereas the flux values without the corrections are shown by dashed diamonds in Fig. 2. The effect of the electrons is estimated to be negligible below 1 MeV.

The present results are compared in the same figure with our earlier results. The filled diamonds are those obtained from a balloon flight made at Hyderabad in 1972 with a similar detector equipped with a plastic guard counter and the open circles are based on a balloon observation from Sanriku, Japan (also in 1972) with a passive collimator and a passive shutter. There is good agreement between the various results in the overlapping energy regions. The spacecraft results by Trombka *et al.*⁵ and the balloon results by Schönfelder and Lichti¹³ are also shown in Fig. 2. These are in general agreement with our results although their flux values are somewhat higher.

Our principal conclusion is that the spectrum gradually steepens in the energy range 100–500 keV and then tends to flatten in the MeV region consistent with a hump around 1 MeV. Single power spectra can fit the data points only with confidence

levels lower than 0.001. Detailed description of the experiment and data analysis will be published elsewhere¹³.

We thank the launch crew of the Balloon Facility of the Tata Institute of Fundamental Research and Mr H. Akiyama of Institute of Space and Aeronautical Science, University of Tokyo for support. The work was partially supported by the Japan Society for the Promotion of Science.

Y. FUKADA
S. HAYAKAWA
I. KASAHARA
F. MAKINO
Y. TANAKA*

Department of Physics,
Nagoya University, Nagoya, Japan

B. V. SREEKANTAN
Tata Institute of Fundamental Research, Bombay, India

Received January 20; revised February 17, 1975.

* Now at Institute of Space and Aeronautical Science, University of Tokyo, Tokyo, Japan.

- 1 Metzger, A. E., Anderson, E. C., Van Dilla, M.A. and Arnold, J. R., *Nature*, **204**, 766 (1964).
- 2 Golenetskii, S. V., *et al.*, *Astrophys. Lett.*, **9**, 69 (1971).
- 3 Vette, J. I., Gruber, D., Matteson, J. L. and Peterson, L. E., *Astrophys. J. Lett.*, **160**, L161 (1970).
- 4 Damle, S. V., Daniel, R. R., Joseph, G. and Lavakare, P. J., *Astrophys. Space Sci.*, **14**, 473 (1971).
- 5 Trombka, J. I., *et al.*, *Astrophys. J.*, **181**, 737 (1973).
- 6 Fishman, G. J., *Astrophys. J.*, **171**, 163 (1972).
- 7 Dyer, C. S., and Morfill, G. E., *Astrophys. Space Sci.*, **14**, 243 (1971).
- 8 Danjo, A., *J. Phys. Soc. Japan*, **33**, 890 (1972).
- 9 Daniel, R. R., and Stephens, S. A., *Revs. Geophys. Space Phys.*, **12**, 233 (1974).
- 10 Thompson, D. J., *J. geophys. Res.*, **79**, 1309 (1974).
- 11 Danjo, A., *et al.*, *Space Res.*, **11**, 1373 (1971).
- 12 Horstman, H., and Horstman-Moretti, E., *Nature phys. Sci.*, **229**, 148 (1971).
- 13 Makino, F., *Astrophys. Space Sci.*, (in the press).

Neutrino processes and QSOs

PECULIAR jets of matter emanate from some galactic nuclei and QSOs, including the galaxy M87 and the QSO 3C273. It is generally believed that these jets are matter ejected from the central regions of the object¹. As a result of non-conservation of parity in weak interactions, the neutrino processes associated with gravitational collapse of a galactic nucleus in the presence of a strong magnetic field can lead to ejection of matter from the nucleus in one direction. I suggest that the jets observed in M87 and 3C273 are the result of this mechanism.

The importance of neutrino processes in gravitational collapse depends on the very large mean free path of neutrinos even in extremely dense matter, which enables them to carry away the gravitational potential energy released in the collapse. Because of conservation of magnetic flux associated with the "frozen in" magnetic field, a collapsing object can acquire an intense magnetic field². In the presence of an intense magnetic field a certain fraction of neutrons in the object will be aligned with spins parallel to the magnetic field; in weak processes involving aligned neutrons the neutrino emission is asymmetrical as a result of non-conservation of parity in weak interactions. So the linear momentum carried away by neutrinos is non-zero and must be compensated by a change in linear momentum of the system. The degree spin alignment of neutrons depends on temperature T and the strength of the magnetic field H . An almost complete spin alignment is possible if $\mu H > kT$ (μ is the magnetic moment of the neutron, k the Boltzmann constant). For a temperature of the order of 10^8 K, this condition is satisfied if $H \sim 10^{16}$ gauss. Optical and radio observations probably rule out the existence of magnetic fields of this strength in the outer regions of a galactic nucleus or a QSO. But the occurrence of magnetic fields of this strength in the central collapsing regions of the nucleus, where the neutrino processes are important, cannot be ruled out. Ginsburg³ has shown that the magnetic field of a collapsing object

can increase without limit. There are also arguments which suggest that highly collapsed matter is 'ferromagnetic' with spins of neutrons aligned in one direction⁴. If we assume almost complete spin alignment, the neutrino angular distribution function $W(\theta)$ is proportional to $(1 + \alpha \cos \theta)$, where θ is measured from the direction of the magnetic field (for nuclear β decay, α is of the order 0.4). If N neutrinos are emitted with average energy E a simple calculation gives the momentum ΔP carried away by neutrinos as

$$\Delta P = (2NE \alpha / 3c) \mathbf{n} \quad (1)$$

where $\mathbf{n}$ is unit vector in the direction of the magnetic field. Thus if a mass δM is converted into neutrinos, by setting $NE = \delta Mc^2$ in equation (1) the momentum carried away by neutrinos is

$$\Delta P = 2/3 (\delta M c \alpha) \mathbf{n} \quad (2)$$

So to conserve linear momentum the object should either recoil or eject matter in the opposite direction. For the mechanism to be effective a large fraction of the material of the nucleus must be in the form of neutrons free to decay. This requirement can probably be realised. In the central regions of a collapsing object matter should exist predominantly in the form of neutrons since at high pressures it is thermodynamically more favourable for protons to capture electrons. The presence of few per cent of electrons will ensure that neutrons are stable against β decay. But there is no reason to believe that the Fermi energy of these electrons will remain the same throughout the collapse. A sudden decrease in Fermi energy will allow neutrons to decay. The quantity δM in equation (2) can be a considerable fraction of the mass of the object. Since α is not very different from unity, large amounts of matter can be ejected in a direction opposite to $\mathbf{n}$ with relativistic speeds. The energy released during neutron decay could temporarily convert the contraction into an expansion. Repetition of this process will also occur but in every contraction phase ejection of matter will take place in the same direction because of the presence of the "frozen in" magnetic field.

The jets associated with most objects have condensations along their length indicating that several ejections have taken place in the same direction. This is in agreement with the mechanism I propose. It is interesting that no other mechanism could easily explain the occurrence of discrete emissions from the nucleus in the same direction. If the jet formation is a magnetohydrodynamic effect there is no reason why the discrete ejections should not occur with equal probability in a direction parallel as well as anti-parallel to the magnetic axis.

K. TENNAKONE

Department of Physics,
University of Sri Lanka,
Vidyodaya Campus, Nugegoda, Sri Lanka

Received December 30, 1974; revised February 17, 1975.

¹ Baade, W., and Minkowski, R., *Astrophys. J.*, **119**, 221 (1954).

² Ginsburg, V. L., *Dokl. Akad. Nauk SSSR*, **156**, 43 (1964).

³ Ginsburg, V. L., *Quasi-Stellar Sources and Gravitational Collapse* (University of Chicago Press, Chicago, 1965).

⁴ Rice, M. J., *Phys. Lett.*, **29A**, 637 (1969).

because there are clearly defined markers, spectral lines of hydrogen and hydroxyl, in the low noise region of the radio spectrum^{1,2}. Although there may be no single optimum frequency, the number of frequencies that must be considered seems to be quite small.

The number of stars that can be considered as possible homes for communicative civilisations, on the other hand, is very large. Recent studies³⁻⁶ agree that the chances of two civilisations establishing contact are exceedingly small in the absence of time markers that will tell the two civilisations when to search for one another. Here we point out that suitable time markers do exist for binary stars. They are the turning points of the stellar orbits, periastron and apastron.

Single star civilisations would logically transmit signals to binaries at the observation of periastron and apastron. Binary star civilisations would scan single stars at a time $2T$ after periastron and apastron, where $2T$ is twice the signal propagation time between the stars. The length of scan need be no longer than twice the uncertainty in T . Contact signals from different stars would arrive at different times at the binary system because of differences in the value of T for each candidate. This strategy would enable a binary star civilisation to complete a search of nearby stars with a small number of telescopes after only a few orbital revolutions.

The same strategy would be followed for transmissions from one binary civilisation to another. Contact signals would be sent at the time of observed periastron and apastron of the receiving civilisation. Contact signals would be sought at a time $2T$ after local periastron and apastron.

The strategy for a binary civilisation attempting to contact a single star civilisation depends on whether the signals are transmitted isotropically or non-isotropically. Signals transmitted isotropically from a binary at its own periastron and apastron will arrive at each target star at the observation of periastron and apastron. On the other hand, with non-isotropic transmission, individual transmissions must be made to all target stars, necessitating very short transmissions or a very large number of transmitters. A solution to this problem would be to delay transmissions by a time known to both civilisations, that is T . This would preserve the time markers but spread the calls out in time.

It may, on the other hand, be preferable to send the contact signals to single stars at the times when the angular distance between the binary stars as seen from the target star is either a maximum or a minimum. These times are known to both civilisations. Because they depend on the orientation of the orbit with respect to the line joining transmitter and receiver the times are different for each target star. Simultaneous calls to many candidates are therefore avoided by this strategy.

Although the optimum strategy may not be immediately apparent, these considerations limit the possibilities to the point where the search in time is no more difficult than the search in frequency. For any advantage to be gained from these strategies, however, it is necessary that the number of reasonable contact times, multiplied by the duration of the search period required to accommodate errors in distance and in orbital elements, should not exceed the period of the binary star. These strategies therefore require accurate knowledge of stellar parallaxes. The strategy of listening for contact signals from binary stars at the times of observed turning points of the orbit or at the times of maximum angular separation does not require knowledge of the distance on the part of the receiving civilisation, however, so it might be advantageous for us. The transmitting civilisation does need to know the distance in order to determine when to listen for replies, but binary star civilisations should be able to measure stellar parallaxes more accurately than we can because of the long baseline provided by the binary orbit.

There is no shortage of binary star systems that may be the homes of communicative civilisations. Of the 100 nearest stars at least 40 are visual binaries, and a total of more than 50,000 visual binaries are known⁷. The probability of the existence of habitable planets in binary star systems has been examined by

Time markers in interstellar communication

THE feasibility of interstellar radio communication using existing facilities has been established in principle¹. We can communicate with extraterrestrial civilisations whose technological powers are no greater than ours by concentrating radiated power and receiver sensitivity in a narrow band of frequencies and a narrow field of view.

Frequency selection is a problem that seems to be tractable

Dole^{8,9} and Huang^{10,11}. There seems to be no reason why visual binaries should not have habitable planets, provided one or both of the stars is of suitable spectral class and provided their eco-spheres have radii considerably smaller than the separation at periastron.

In the Worley Catalog of Visual Binary Orbits¹² there are 536 systems with known orbits and periastron dates. Of these we have determined that about 300 systems may have habitable planets about one or both components. The number of stars in these systems that may support habitable planets exceeds 500. As an illustration of the possibilities we have prepared a partial list of opportunities for the detection of signals from civilisations associated with visual binaries that we judge to be habitable. Considering only systems closer than 300 light yr we find 34 opportunities between 1975.0 and 1980.0.

If there are communicative civilisations associated with any of the nearby binary stars it should occur to them, as it has to us, to use the orbital motions of their stars as an indicator of when to send and when to look for contact signals. There seems to be a reasonably small number of possibilities. These considerations could lead to a non-random search programme with some chance of success. They could also provide a practical basis on which to initiate our own transmission of contact signals.

We thank Professors D. R. Bates and P. B. Hays for suggestions. Partial support has been provided by NASA. The National Astronomy and Ionosphere Center is operated by Cornell University under contract with the National Science Foundation.

GORDON W. PACE

5718 NE 28th Avenue,
Portland, Oregon 97211

JAMES C. G. WALKER

Arecibo Observatory,
National Astronomy and Ionosphere Center,
Arecibo, Puerto Rico 00612

Received August 5, 1974.

¹ Drake, F. D., and Sagan, C., *Nature*, **245**, 257–258 (1973).

² Cocconi, G., and Morrison, P., *Nature*, **184**, 844–846 (1959).

³ Bates, D. R., *Phys. Bull.*, **23**, 26–29 (1972).

⁴ Walker, J. C. G., *Nature*, **241**, 379–381 (1973).

⁵ Bates, D. R., *Nature*, **248**, 317–318 (1974).

⁶ Bates, D. R., *Nature*, **252**, 432–433 (1974).

⁷ Brown, H., *Science*, **145**, 1177–1181 (1964).

⁸ Dole, S. H., *Habitable Planets for Man* (Elsevier, New York, 1970).

⁹ Dole, S. H., *Icarus*, **13**, 494–508 (1970).

¹⁰ Huang, S.-S., in *Interstellar Communication* (edit. by Cameron, A. G. W.), 93 (Benjamin, New York, 1963).

¹¹ Huang, S.-S., *Icarus*, **18**, 339–376 (1973).

¹² Worley, C., *A Catalog of Visual Binary Orbits* (US Government Printing Office, Washington, 1964).

7 to 10 °C; at 50 km it is 5 °C, and below 40 km the error is less than 3 °C.

Time-height cross sections of the mesospheric temperatures in the region 50 to 80 km are plotted using the conventional interpolation method in Fig. 1. Meridional and zonal temperature gradients are derived for altitudes of 65, 70 and 75 km from the corresponding upper wind and temperature results using the thermal wind equations

$$\frac{\partial T}{\partial y} = \frac{fT}{g} \left(\frac{\partial u}{\partial z} - \frac{u}{T} \frac{\partial T}{\partial z} \right)$$

$$\frac{\partial T}{\partial x} = \frac{-fT}{g} \left(\frac{\partial v}{\partial z} - \frac{v}{T} \frac{\partial T}{\partial z} \right)$$

where f is the coriolis parameter, g the acceleration due to gravity, T the absolute temperature at a particular level, x , y , z the eastward, northward and vertical axes; u , v the zonal and meridional components of winds with west and south positive, respectively. The horizontal temperature gradients are given in Table 1.

Time sections of the upper atmospheric temperatures showing annual variations at the altitudes 30, 50 and 70 km as derived from the successful 1972 rocket soundings are plotted in Fig. 2. Corresponding upper atmospheric warming and cooling of more than 15 °C over periods of less than 15 d are given in Table 2 for 5-km altitude intervals during the winter period May to August and early spring.

Figure 1 and Table 1 are confined to the mesosphere only, whereas Fig. 2 and Table 2 extend the study to the stratosphere. Figure 1 shows that in the southern summer months January–February the mesospheric temperatures in the region 50 to 80 km ranged from about 10 to –110 °C, with the summer maximum reached by about January 5 in the lower mesosphere and by about January 26 in the upper mesosphere. The disruption of the mesospheric winter regime by a period of stormy winds¹ is clearly shown in Fig. 1. In the third week of May and the first week of July the upper mesosphere was subjected to a warming of more than 30 °C in less than a week. The temperature distribution returned to normal by early September, but was again disrupted in about the third week.

Table 1 shows that the horizontal temperature gradients at 65 km ranged from –37 to 10 °C per 5° latitude with a maximum on September 6. At 70 km the range was –31 to 30 °C with the maximum values on June 28 and May 17, whereas the gradients at 75 km ranged from –14 to 21 °C per 5° latitude with the maxima on September 6 and May 17, respectively. Larger gradients were found from May to July, indicating a disruption by stormy winds having large wind shears of about $10 \times 10^{-3} \text{ s}^{-1}$ to $30 \times 10^{-3} \text{ s}^{-1}$. A similar disruption occurred in September. The negative meridional temperature gradients represent a decrease of the zonal winds with altitude (the easterlies increase and the westerlies decrease with height) and the negative zonal temperature gradients represent an increase of the meridional winds with altitude (the northerlies decrease and the southerlies increase with height). On May 17 at 70 km the meridional temperature gradient was 30.4, suggesting a disruption by strong easterlies which decreased with height.

Curve *C* in Fig. 2 shows that at 30 km the stratospheric temperatures in Antarctica fall as the Sun sets, and that the minimum –75 °C is reached in June. A slow temperature rise begins as the Sun returns, but this becomes much more marked after early September when the Sun's radiation reaches the lower strata. Curve *B* (50 km, around the boundary of the stratosphere and the mesosphere) shows a warming of 23 °C during the second week of May followed by an equal cooling during the third week. During the second week of July there was a mid-winter stratospheric warming of 30 °C. The temperatures at 50 km ranged from a maximum of 12 °C in early

Upper atmospheric thermal structure in Antarctica

THE mesospheric wind results derived for atmospheric altitudes between 50 and 90 km from meteorological rocket flights carried out at Molodezhnaya station (67° 40' S, 45° 51' E) in Antarctica have been discussed in ref. 1. The rapid shifts in both zonal and meridional components of the winds during May to July indicated a sudden 'explosive' change in temperature distribution in the upper mesosphere over Antarctica in the winter regime. I have investigated this phenomenon and extended the study down to the stratosphere; the results are discussed here. The meridional and the zonal temperature gradients at altitudes of 65, 70 and 75 km, derived from the thermal wind equations using the corresponding upper wind and temperature results, have also been studied. Atmospheric temperatures up to an altitude of about 80 km were also measured and the accidental root mean square errors in the determinations were as follows: in the altitude region 60 to 80 km it does not exceed

Table 1 Meridional and zonal temperature gradients at Molodezhnaya, Antarctica, in 1972

Date	65 km		70 km		75 km	
	Temperature gradient*		Temperature gradient*		Temperature gradient*	
	Meridional	Zonal	Meridional	Zonal	Meridional	Zonal
January 5	0.3	-2.1	-6.3	-0.8	-1.7	3.1
January 19	-2.0	-0.6	0.3	-6.5	2.5	1.4
January 26	0.8	3.0	-1.7	2.6	10.6	-1.7
February 16	-9.4	-0.3	3.3	3.5	-5.0	-5.9
May 3	-9.3	-4.0	-5.0	17.8	-1.0	-2.1
May 17	-20.2	-8.1	30.4	-0.5	-4.8	20.9
June 28	5.2	2.3	-31.3	10.5	-7.1	5.5
July 5	-13.4	-7.8	-20.9	6.9	-7.5	-1.0
July 19	-10.4	-3.7	8.2	5.6	9.1	1.9
September 6	-36.7	9.9	-1.1	-1.7	9.1	-14.1
October 4	-0.6	-3.8	-5.4	5.4	2.7	-2.2
December 20	0.1	0.7	4.2	-3.4	-0.2	3.7

* °C per 5° latitude.

December to a minimum of -45°C at the end of May. Curve *A* (70 km) reveals sudden 'explosive' changes in the upper mesospheric temperature distribution, particularly during the winter period May to July. During the third week of May, a warming of 26°C occurred, and in midwinter there was a warming of 31°C during the first week of July followed by a 'sudden cooling' of 53°C during the second week. The temperatures at 70 km show a distinct maximum of -12°C during the first week of July, after which the temperature declines irregularly, attaining a minimum of -93°C in mid-November. Again in September there was a disruption with a warming of 20°C followed by a cooling of 33°C after which

the temperature distribution returned to the normal summer regime.

Table 2 reveals that in May there was a significant warming both in the upper stratosphere and the mesosphere with a 'sudden warming' of 49°C at 73 km from May 17 to 24. The warming was followed by a cooling of about 30°C in the lower mesosphere. From June 28 to July 5, a warming of 37°C was detected at 65 km. The upper stratosphere and the lower mesosphere were subjected to a warming from July 5 to 19, with a maximum of 38°C at 55 km, whereas the upper mesosphere underwent a 'sudden cooling' which had a maximum value of 55°C at 65 km from July 5 to 12. In August and

Fig. 1 Time-height cross section of the mesospheric temperatures ($^{\circ}\text{C}$) at Molodezhnaya, Antarctica in 1972. Dashed lines, extrapolated data. Arrows above the abscissa show the dates on which the data were obtained.

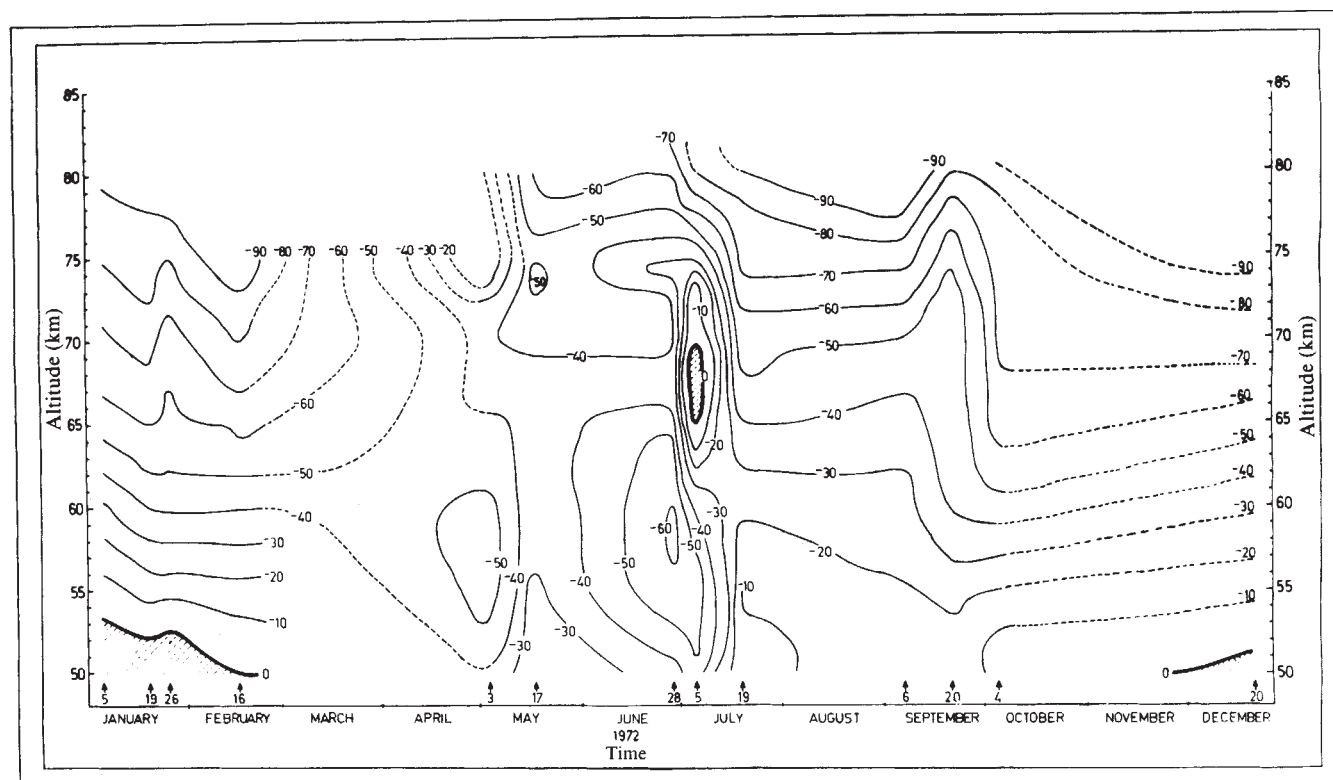


Table 2 Upper atmospheric warming and cooling of greater than 15 °C over periods less than 15 d at Molodezhnaya, Antarctica, in 1972

Altitude (km)	May		June-July		July		July-August		August		August-September		September	
	Warming	Cooling	Warming	Warming	Cooling	Warming	Cooling	Warming	Cooling	Warming	Warming	Cooling	Warming	Cooling
35				22*					27				38	
40	18			(5-12)†					(9-16)				(6-20)	
45	(10-17)			29			21		32				39	-20
50	21	-18		(5-12)		(26-2)			(9-16)	(2-9)			(6-20)	(20-27)
55	(10-17)	(3-10)		19					-22				17	
60	26	-32		(5-12)					(16-23)				(13-20)	
65	(3-17)	(17-24)		31					-26	21				
70	24	-27		(5-19)			-23		(9-23)	(23-6)				
75	(10-17)	(17-24)		38			(26-2)			19				-26
80	22	-28	19	(5-19)			-26			(23-6)				(13-27)
	(10-17)	(17-31)	(28-5)	20			(26-2)	18		15				-27
	36			(12-19)			(26-2)	(20-23)		(23-6)				(20-27)
	(20-24)		(28-5)		-55		-18	22					19	-22
	26				(5-12)		(26-2)						(13-20)	(20-27)
	(20-24)		(28-5)		-53								20	
	49				(5-12)			16					(13-20)	
	(17-24)				-48			(6-20)					16	
	29				(5-12)								(13-20)	
	(17-24)				-25									
	36	-43			(22-26)									
	(17-20)	(20-24)			-39	22								
					(22-26)	(26-2)								

*°C.

†Dates.

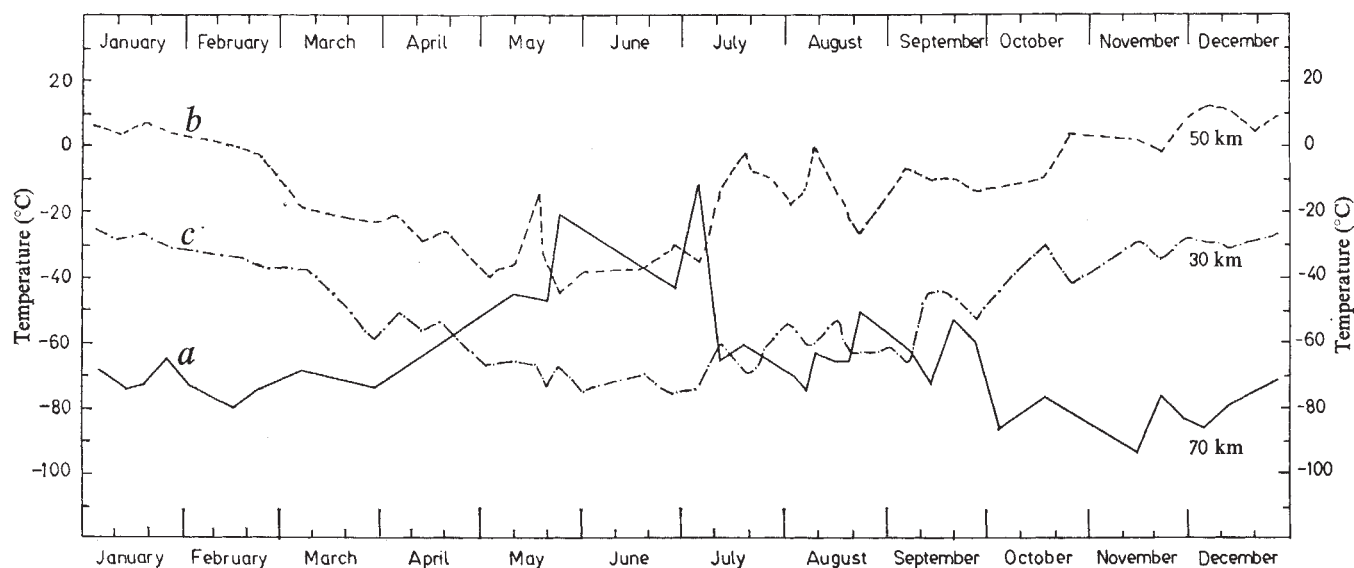
September the warming in the mesosphere was less than 27 °C, while the stratosphere at 40 km experienced a cooling of 26 °C from August 2 to 9, followed by a warming of 32 °C from August 9 to 16. At 40 km there was another warming of 39 °C from September 6 to 20, which was followed by a cooling of 20 °C from September 20 to 27.

This investigation of the Antarctic upper atmosphere indicates that the most active period in south polar regions is the winter and the early spring: it is marked by large disruptions in the wind and thermal structure. The rapid shifts in both zonal and meridional components of the upper atmospheric winds, particularly during the winter period May to July, were accompanied by sudden changes in the temperature distribution as revealed by warming and cooling in Fig. 2.

Figure 2 shows that the stratospheric warming in May propagated upwards and was followed by the mesospheric warming. The stratospheric warming in July followed the

mesospheric warming, indicating downward propagation of the disturbance which started above 70 km. This leads to the intuitive conclusion that the polar winter warmings may be caused both by an increase in the supply of energy in the form of a vertical flux of geopotential energy consisting of very long waves, and by radiative and photochemical processes taking place in the upper atmosphere. During September, when the winter westerlies changed to the summer easterlies, the upper atmosphere was again disrupted with a warming of 39 °C at 40 km which is attributed to the increase in available heat brought about by the return of sunlight. It is thus concluded that sizeable perturbations may occur in the upper atmosphere over Antarctica during the winter regime.

I acknowledge the collaboration of the members of the Soviet Antarctic Expedition, 1971-73, and thank the Department of Atomic Energy, Government of India and the Hydro-meteorological Service of the USSR for allowing me to take

Fig. 2 Time section of annual variation of the upper atmospheric temperatures (°C) for the altitudes 30, 50 and 70 km at Molodezhnaya, Antarctica, in 1972.

part in the expedition, and giving me the privilege of being the first Indian explorer of Antarctica, which was made possible by the late Professor Vikram A. Sarabhai. I thank Professor P. R. Pisharoty for guidance and discussions. I also thank my parents for inspiration.

PARMJIT SINGH SEHRA*

Physical Research Laboratory,
Ahmedabad 380 009, India

Received January 13, 1975.

*Also at: Remote Sensing and Meteorology Applications Division, Space Applications Centre, Indian Space Research Organisation, Ahmedabad, India.

¹ Sehra, P. S., *Nature*, 252, 683 (1974).

Phlogopite stability and the $^{87}\text{Sr}/^{86}\text{Sr}$ step in basalts along the Reykjanes Ridge

THE relatively sharp step in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio within basalts midway along the Reykjanes Ridge may reflect the breakdown of titaniferous phlogopite during variable fusion of an essentially homogeneous phlogopite-lherzolite upper mantle, extending beneath both Iceland and the submarine parts of the Mid-Atlantic Ridge.

Schilling¹ detected substantial variation in the concentrations of several elements within basalts collected along the Reykjanes Ridge, south of Iceland. To explain this he proposed two magma sources; a primordial hot mantle plume, relatively enriched in large-ion lithophile (LIL) trace elements and radiogenic Sr and Pb isotopes, rising beneath Iceland, and relatively LIL-depleted upper mantle within the low velocity layer beneath the Mid-Atlantic Ridge. Mixing of these sources and/or their partial melt products was postulated to produce the chemical variation observed along the Reykjanes Ridge. Subsequent work^{2,3} showed that the transition from Icelandic (0.70304) to Mid-Atlantic Ridge (0.70270) values for the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio in basalts was not by a smooth gradation but by a step confined to a zone 200–250 km south of the Reykjanes Peninsula. Several other trace elements behave similarly. Critics of the two-mantle-source model^{4,5} have emphasised that most Icelandic basalts (some of which approach tholeiitic andesite compositions) are very strongly fractionated relative to the compositions of magmas that could conceivably have been generated by partial fusion of any postulated upper mantle material. The rare-earth element (REE) patterns reported by Schilling¹ may be fitted adequately to a model invoking variable partial fusion of a single source⁶.

The proposals of Schilling's critics do not account for two crucial observations: first, the varying Sr and Pb isotopic ratios in the lavas; second, the high rate of magma production in Iceland relative to deep-sea sections of the Mid-Atlantic Ridge, as evidenced by thickening of the crust beneath the former⁶. We are reluctant as yet to accept the two-mantle-source model because it is essentially defeatist. It may eventually be proved that the two-mantle-source model is correct, but meanwhile the single-mantle alternative should continue to be considered. We concentrate here on $^{87}\text{Sr}/^{86}\text{Sr}$ variation in the basalts, perhaps the most persuasive evidence for the two-mantle-source model, and attempt to show that it is not irreconcilable with a single mantle composition.

$^{87}\text{Sr}/^{86}\text{Sr}$ in basalts from the transition zone between the relatively isotopically-homogeneous lava groups of Iceland and the Mid-Atlantic Ridge shows a good correlation with the water depth at each collection site (Fig. 1 and ref. 3). This reflects local topographical irregularities in the Reykjanes Ridge and is not merely a result of the overall deepening of the Atlantic south of

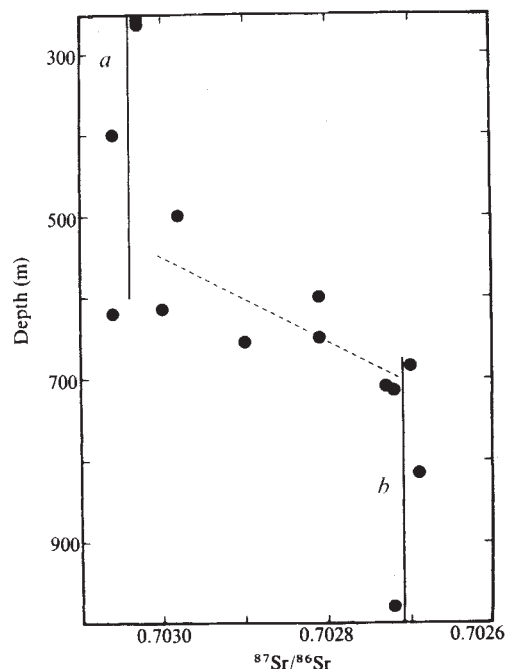


Fig. 1 $^{87}\text{Sr}/^{86}\text{Sr}$ of Reykjanes Ridge basalts as a function of the water depth from which they were dredged^{1,3}. The Iceland and Mid-Atlantic Ridge average values³ of $^{87}\text{Sr}/^{86}\text{Sr}$ extend for a very much greater range of water depths (~0–2,500 m) along the axis of the North Atlantic than that covered by this diagram. *a*, Mean $^{87}\text{Sr}/^{86}\text{Sr}$ for Iceland; *b*, mean for Mid-Atlantic Ridge.

Iceland; there is little correlation of $^{87}\text{Sr}/^{86}\text{Sr}$ between the points in the transition zone in Fig. 1 and their distance from the tip of the Reykjanes Peninsula. Hart *et al.*³ attribute this relationship to dynamic interfingering between their two postulated mantle types. This proposal seems improbable when the high postulated viscosity of the upper mantle (~ 10^{22} poise) at appropriate pressure and temperature⁷ is compared with the small scale of topographical⁸ and isotopic variation in the transition zone; lavas with distinctively different $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are found as little as 2 km apart³.

There are two alternative explanations of the $^{87}\text{Sr}/^{86}\text{Sr}$ –depth relationship, both coming from discoveries^{9,10} that volcanism on the Mid-Atlantic Ridge is essentially confined to the axis of the structure and that, as the European and American plates separate at a constant rate (on the spatial and temporal scale considered here), the thickness of the ridge lava pile, monitored by water depth, is a direct measure of the rate of magma production beneath each ridge segment. It follows that:

(1) If the system is constrained by assuming constant mantle circulation rates beneath both topographic highs and lows on the Reykjanes Ridge, a greater amount of mantle partial fusion must have occurred beneath the highs than beneath the lows during basalt genesis. So the relative ^{87}Sr -enhancement of lavas from the highs may reflect the thermal breakdown of an ^{87}Sr -rich mineral in the upper mantle. This phase would remain in the residuum during production of the magmas which give rise to the Mid-Atlantic Ridge basalts and only melt during more intense fusion beneath Iceland and the northern Reykjanes Ridge.

(2) If the driving force behind the movement of separating lithospheric plates is independent of mantle turnover beneath mid-oceanic ridges¹¹, the mantle passively inflowing between them as they part could move at very different rates in different localities. If mantle fusion is related to its flow rate through heat generation resulting from viscous dissipation⁷, the topographical heights on the Reykjanes Ridge may simply reflect zones of relatively rapid mantle creep beneath them. The degree of partial

melting involved in the production of each magma batch might in this case be the same beneath both the Mid-Atlantic Ridge and Iceland. Indeed, it may be even less beneath the latter, if enhanced kneading within fast-flowing mantle causes a positive correlation between flow rate and efficiency of magma extraction during partial fusion¹². In this case it is possible that magma generation takes place at greater depths beneath Iceland than the Mid-Atlantic Ridge. The $^{87}\text{Sr}/^{86}\text{Sr}$ step in Reykjanes Ridge basalts may mark the breakdown of an ^{87}Sr -rich mantle mineral with increasing pressure northwards at the site of partial fusion. If the Reykjanes Ridge crust is extremely weak, as seems probable from what is known of the strength of thin hot crust elsewhere¹³, its topographical irregularities may give rise to small local pressure differences (< 50 bar, see Fig. 1) at the level of partial melting below. The $^{87}\text{Sr}/^{86}\text{Sr}$ -depth correlation within the transition zone in Fig. 1 would then reflect passage of this ^{87}Sr -rich mantle phase in and out of its stability field along irregularities in the isobar marking its maximum stability at depth.

Is there a likely upper mantle phase with the chemistry and PT stability to satisfy any parts of the genetic models discussed above? We suggest that phlogopite is the mineral responsible for the $^{87}\text{Sr}/^{86}\text{Sr}$ step. It has been postulated frequently as a minor phase in the upper mantle¹⁴⁻¹⁹ and the suggestion has been made in two recent papers^{20, 21} that fusion of phlogopite might be important in controlling K, Rb, Ba and ^{87}Sr contents of Icelandic and Mid-Atlantic Ridge basalts. But neither discusses the PT stability of phlogopite relative to other likely phases in the upper mantle.

Experimental studies of the stability of synthetic Ti-free phlogopite^{18, 19, 22} have indicated that, if undersaturated with water at moderate pressures, it would probably be stable up to the solidus of a peridotite upper mantle but would enter the first fraction of liquid formed. But the phlogopite that occurs as a primary phase in generally-accepted xenolithic samples of the upper mantle, such as garnet-lherzolite blocks in rapidly erupted volcanic rocks¹⁸, frequently has a substantial Ti content ($< 9\%$ TiO_2). Synthetic Ti-phlogopite [$\text{K}_2\text{Mg}_4\text{TiAl}_2\text{Si}_6\text{O}_{20}(\text{OH})_4$] has a considerably greater thermal stability at all in-

vestigated pressures than its Ti-free analogue²³. Even making allowance for the presence of other mantle phases (extrapolated from the Ti-free phlogopite data), it seems probable (Fig. 2) that Ti-phlogopite would remain in the residuum after fusion of a considerable fraction of a peridotite upper mantle at pressures below about 10 kbar. Only at higher pressures would Ti-phlogopite enter near-solidus melts.

The data of Fig. 2 may be applied to upper mantle fusion beneath Iceland and the Mid-Atlantic Ridge, if the geothermal gradients in these areas are known. The latter are not easily extrapolated from surface heat-flow measurements in regions of active volcanism, because of magma movement near the surface, and geothermal water circulation. Experimental petrology is an alternative way of approaching the problem. Kushiro and Thompson²⁴ studied the melting behaviour of Mid-Atlantic Ridge lavas as a function of pressure. One of the most Mg-rich of the phenocryst-poor basalts ($\text{MgO} = 9.17\%$) as yet known from the Ridge shows liquidus coprecipitation of three phases (olivine + pigeonite + plagioclase) at 7.5 kbar. The simplest interpretation of this phenomenon²⁴ is that the basalt formed by fusion of a plagioclase-lherzolite upper mantle at 7.5 kbar and was erupted without significant further modification. Fe/Mg of phases near liquidus in the basalt indicate a residual mantle, after Mid-Atlantic Ridge basalt extraction, with $\text{Mg}/(\text{Mg} + \text{Fe}) \sim 0.85$, in accordance with estimates from other volcanic areas^{11, 25}. Acceptance of a primary magma hypothesis for this lava allows calculation of a Mid-Atlantic Ridge geothermal gradient, fitted to the liquidus temperature ($1,215^\circ\text{C}$) at the four-phase point. The result of 45°C km^{-1} overall corresponds well with previous estimates by other methods^{26, 27}. Unfortunately, there are no comparable experimental data available as yet to allow calculation of Icelandic geotherms.

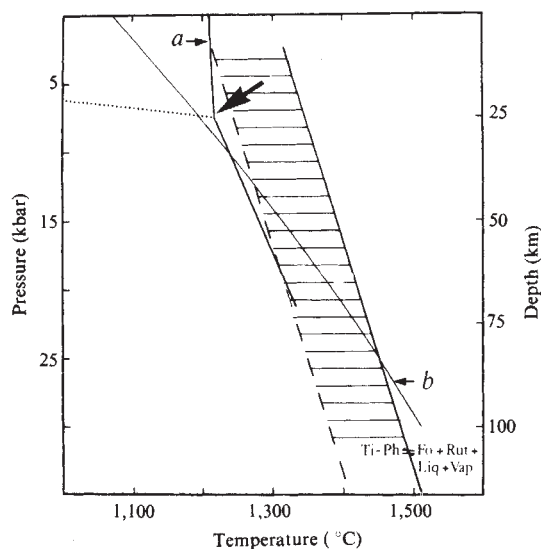
If the geotherm below the Mid-Atlantic Ridge reaches $1,215^\circ\text{C}$ at about 7.5 kbar, it is appreciably above the anhydrous solidus at that pressure of either a pyrolite²⁸ or a $\text{Mg}/(\text{Mg} + \text{Fe}) = 0.85$ lherzolite²⁹ upper mantle (Fig. 2). But it is approximately at, or slightly below, the extrapolated stability limit of Ti-phlogopite.

The two models for the $^{87}\text{Sr}/^{86}\text{Sr}$ step in basalts from the Reykjanes Ridge may now be reassessed:

(1) If the degree of mantle partial fusion during magma genesis increases northwards beneath the Reykjanes Ridge, then the $^{87}\text{Sr}/^{86}\text{Sr}$ step in Fig. 1 and its correlation with thickness of lava pile may reflect the isobaric breakdown of titaniferous phlogopite in the source of the basalts. This proposal leads to the surprising conclusion that the relative enrichment of Icelandic basalts in the 'incompatible' elements K, Rb, Cs, Ti, Ba and ^{87}Sr (ref. 28) may not be because they originate as lesser fractions of mantle fusion than Mid-Atlantic Ridge basalts. At first sight this seems highly improbable, because light REE and P are also concentrated in the Icelandic lavas. But phlogopite can be relatively enriched in the light rare-earth elements³⁰ and its breakdown could contribute these elements to the melt. Furthermore, concomitant REE and P variation may reflect the stability relations of apatite in the upper mantle. The behaviour of this phase during partial fusion may be as complex as that postulated here for phlogopite. Until this is known, or until data are published for LIL elements that are not concentrated in apatite or phlogopite, it seems premature to reject this model for the chemical variation in the Reykjanes Ridge basalts.

(2) If the depth of the partial fusion zone increases northwards, the $^{87}\text{Sr}/^{86}\text{Sr}$ step beneath the Reykjanes Ridge may mark the intersection of this zone with the Ti-phlogopite breakdown curve. In this model the degree of partial melting could decrease towards Iceland, so that apatite might then be envisaged as entering the first melt fraction. An attractive feature of this alternative is apparent when it is applied to the general problem of the chemical differences between mid-oceanic ridge and ocean island lavas, of which the Iceland-Mid-Atlantic Ridge system is just a facet. Ocean islands forming away from the crests of mid-oceanic ridges are composed, in general, of lavas less saturated in Si than those erupted at spreading axes^{17, 31, 32}. The available

Fig. 2 P - T diagram correlating the following data: anhydrous solidus of pyrolite²⁸, a possible model composition for the upper mantle; anhydrous liquidus of Mid-Atlantic Ridge magnesian basalt T-87 (ref. 24); upper limit of thermal stability, in conditions free from vapour, of titaniferous phlogopite and extrapolation of this to stability in the presence of other likely upper mantle minerals²³, "T-87 Geotherm" (see text); — — —, extrapolated stability; a, T-87 liquidus; b, anhydrous pyrolite solidus. Arrow marks four-phase point.



experimental data^{29,33} indicate that partial fusion of a lherzolite upper mantle at less than 8 kbar, as postulated earlier for Mid-Atlantic Ridge basalts, could not yield nepheline-normative magmas, however low the degree of partial melting. The latter have probably been generated at pressures above about 15 kbar (refs 25, 29, 33).

The largest potential weakness in all the foregoing argument concerns ⁸⁷Sr/⁸⁶Sr equilibration amongst mantle phases. This must be assumed not to occur either before or during partial fusion, if enrichment of magmas with ⁸⁷Sr is to be linked with phlogopite stability. Most ⁸⁷Sr in phlogopite-lherzolite mantle will be generated from Rb contained in the phlogopite. Isotopic studies of the minerals within peridotite xenoliths from sub-continental upper mantle sources indicate approximate equilibrium³⁴ in phlogopite-bearing garnet-lherzolites and disequilibrium within spinel-lherzolites^{35,36}. The relevance of data derived from material originating in continental parts of lithospheric plates to conditions in the sub-oceanic upper mantle requires further study. At present we strongly support the geochemically based model of O'Nions and Pankhurst²¹, which postulates lack of isotopic equilibration amongst the solid phases of a homogeneous phlogopite-lherzolite upper mantle, both before and during its partial fusion at sites of magma genesis, as the cause of ⁸⁷Sr/⁸⁶Sr (amongst other) differences between Mid-Atlantic Ridge and Atlantic Ocean Island lavas.

I. L. Gibson, M. J. O'Hara and P. Suddaby made detailed constructive criticisms of the manuscript. M.F.J.F. and H.-U.S. received support from the Deutsche Forschungsgemeinschaft.

Note added in proof: Our attention has been drawn to a paper by M. J. O'Hara, M. J. Saunders and E. L. P. Mercy (*Phys. Chem. Earth*, in the press) which contains ideas very similar to ours on the postulated major role of upper mantle phlogopite in controlling ⁸⁷Sr contents of basalts.

M. F. J. FLOWER

H.-U. SCHMINCKE

Institut für Mineralogie der Ruhr-Universität,
463 Bochum, West Germany

R. N. THOMPSON

Department of Geology,
Imperial College of Science and Technology,
London SW7 2AZ, UK

Received December 18, 1974; accepted January 18, 1975.

- ¹ Schilling, J.-G., *Nature*, **242**, 565-571 (1973).
- ² Hart, S. R., and Schilling, J.-G., *Yb. Carnegie Inst. Washington*, **72**, 259-262 (1973).
- ³ Hart, S. R., Schilling, J.-G., and Powell, J. L., *Nature phys. Sci.*, **246**, 104-107 (1973).
- ⁴ O'Hara, M. J., *Nature*, **243**, 507-508 (1973).
- ⁵ Treuil, M., and Varet, J., *Bull. Soc. géol. France*, **15**, 506-540 (1973).
- ⁶ Palmason, G., *Soc. Sci. Isl.*, **40**, 1-187 (1971).
- ⁷ Shaw, H. S., *J. Petrol.*, **10**, 510-535 (1969).
- ⁸ Talwani, M., Windisch, C. C., and Langseth, G., Jr., *J. geophys. Res.*, **76**, 473-517 (1971).
- ⁹ Moore, J. G., Fleming, H. S., and Phillips, J. D., *Geology*, **2**, 437-440 (1974).
- ¹⁰ Hekinian, R., *J. geol. Soc. London* (in the press).
- ¹¹ Shaw, H. S., and Jackson, E. D., *J. geophys. Res.*, **78**, 8634-8652 (1973).
- ¹² Weertman, J., *Bull. Geol. Soc. Am.*, **83**, 3531-3532 (1972).
- ¹³ Walcott, R. I., *J. geophys. Res.*, **75**, 3941-3954 (1970).
- ¹⁴ Kushiro, I., Syono, Y., and Akimoto, S., *Earth planet. Sci. Lett.*, **3**, 197-203 (1967).
- ¹⁵ Dawson, J. B., and Powell, D. G., *Contr. Min. Petrol.*, **22**, 233-237 (1969).
- ¹⁶ Dawson, J. B., Powell, D. G., and Reid, A. M., *J. Petrol.*, **11**, 519-548 (1970).
- ¹⁷ Flower, M. F. J., *Contr. Min. Petrol.*, **32**, 126-137 (1971).
- ¹⁸ Modreski, P. J., and Boettcher, A. L., *Am. J. Sci.*, **272**, 852-869 (1972).
- ¹⁹ Modreski, P. J., and Boettcher, A. L., *Am. J. Sci.*, **273**, 385-414 (1973).
- ²⁰ Sigvaldason, G. E., Steinthorsson, S., Oskarsson, N., and Imsland, F., *Nature*, **251**, 579-582 (1974).
- ²¹ O'Nions, R. K., and Pankhurst, R. J., *J. Petrol.*, **15**, 603-634 (1974).
- ²² Yoder, H. S., Jr., and Kushiro, I., *Am. J. Sci.*, **267A**, 558-582 (1969).
- ²³ Forbes, W. C., and Flower, M. F. J., *Earth planet. Sci. Lett.*, **22**, 60-66 (1974).
- ²⁴ Kushiro, I., and Thompson, R. N., *Yb. Carnegie Inst. Washington*, **71**, 403-406 (1972).
- ²⁵ Thompson, R. N., *Contr. Min. Petrol.*, **45**, 317-341 (1974).
- ²⁶ Turcotte, D. L., and Oxburgh, E. R., *J. Fluid. Mech.*, **28**, 29-42 (1967).
- ²⁷ Bottlinga, Y., *Tectonophysics*, **21**, 15-38 (1974).
- ²⁸ Green, D. H., and Ringwood, A. E., *Earth planet. Sci. Lett.*, **3**, 151-160 (1967).
- ²⁹ Kushiro, I., *Tectonophysics*, **17**, 211-222 (1973).
- ³⁰ Ridley, W. I., and Dawson, J. B., *Abs. Int. Conf. Kimberlites, Cape Town*, 277-278 (1973).
- ³¹ Gast, P. W., *Geochim. cosmochim. Acta*, **32**, 1057-1086 (1968).
- ³² Schmincke, H.-U., and Flower, M. F. J., *Naturwissenschaften*, **61**, 288-297 (1974).
- ³³ O'Hara, M. J., *Earth Sci. Rev.*, **4**, 69-133 (1968).
- ³⁴ Barrett, D. R., *Abs. Int. Conf. Kimberlites, Cape Town*, 19-21 (1973).
- ³⁵ Paul, D. K., *Contr. Min. Petrol.*, **34**, 22-28 (1971).
- ³⁶ Stueber, A. M., and Ikramuddin, M., *Geochim. cosmochim. Acta*, **38**, 207-216 (1974).

Palaeogeotherms and the diopside-enstatite solvus

THE precise composition of the solid solutions that develop between diopside and enstatite has provided a basis for estimates of the temperature of equilibration of mineral assemblages from peridotites. We report here results of a reinvestigation of these relationships, which demonstrate that the range of solid solutions formed at high temperatures is less than previously believed, and that it is dependent on both pressure and silica activity. The stable existence of an iron-free pigeonite has not been confirmed.

Garnet-lherzolite xenoliths in kimberlite are widely accepted as xenoliths of the upper mantle. They contain olivine, garnet and enstatite coexisting with a clinopyroxene. They can be divided into two groups; in one the clinopyroxene is calcic and in the other it is subcalcic. The distribution of analyses of the clinopyroxenes is bimodal¹.

Temperatures and pressures of equilibration of the mineral assemblages in individual nodules from the xenoliths have been estimated². The palaeogeotherms which existed in the upper mantle immediately before incorporation and transport of the xenoliths by kimberlite eruptions have been derived from data from large numbers of individual xenoliths². These geotherms show an abrupt rise in the rate of increase of temperature with depth, coincident with the change in clinopyroxene composition from calcic to subcalcic diopside.

The temperature estimates were based on a comparison of the Ca/Ca+Mg ratio of the diopside coexisting with enstatite in the natural rocks, with the Ca/Ca+Mg ratio of diopside coexisting with enstatite in the system $\text{CaMgSi}_2\text{O}_6\text{-Mg}_2\text{Si}_2\text{O}_6$, as determined by experiments at 30 kbar (ref. 3). The procedure assumed this relationship to be independent of pressure. The results on the synthetic system indicate that enstatite is in equilibrium with a single clinopyroxene phase over the whole temperature range investigated and that the composition of that diopside solid solution changes continuously with temperature. The rate of change of the diopside composition did not, however, vary regularly, and reached a maximum at temperatures close to 1,450 °C (ref. 3). A study⁴ of the same system at 20 kbar reported an abrupt change of the clinopyroxene coexisting with enstatite at this same temperature, from a calcic diopside at lower temperatures to a pigeonite at higher temperatures. Above 1,450 °C a further solvus was reported between the pigeonite and a more calcic diopside. The composition-temperature field occupied by a single clinopyroxene solid solution in the results reported³ at 30 kbar is thus occupied by three fields, of clinopyroxene A (diopside), clinopyroxene B (pigeonite) and clinopyroxene A plus clinopyroxene B in the results reported⁴ at 20 kbar.

The possibility that the abrupt change from diopside to pigeonite in equilibrium with enstatite might have a bearing on the abrupt change from calcic to subcalcic diopside in the xenoliths from kimberlite, and on the change in slope of the inferred palaeogeotherms, prompted this reinvestigation of the clinopyroxene limb of the solvus at 20 and 30 kbar; the results show that pigeonite is not an equilibrium product in this system at those pressures. The position of the equilibrium diopside limb of the solvus at high temperatures is very different from that observed by Davis and Boyd³. Coexisting diopside and enstatite solid solutions do not have the same Si/Ca+Mg ratio, that is, the join $\text{CaMgSi}_2\text{O}_6\text{-Mg}_2\text{Si}_2\text{O}_6$ cuts across the tie lines linking coexisting diopside and enstatite solid solutions which depart, in opposite senses, from the ideal 1:1 ratio of Si/Ca+Mg. The position of the diopside limb is, moreover, pressure dependent.

The discrepancies between these and previous results are predominantly a function of the nature of the starting material. Results obtained from glass starting materials yield metastable

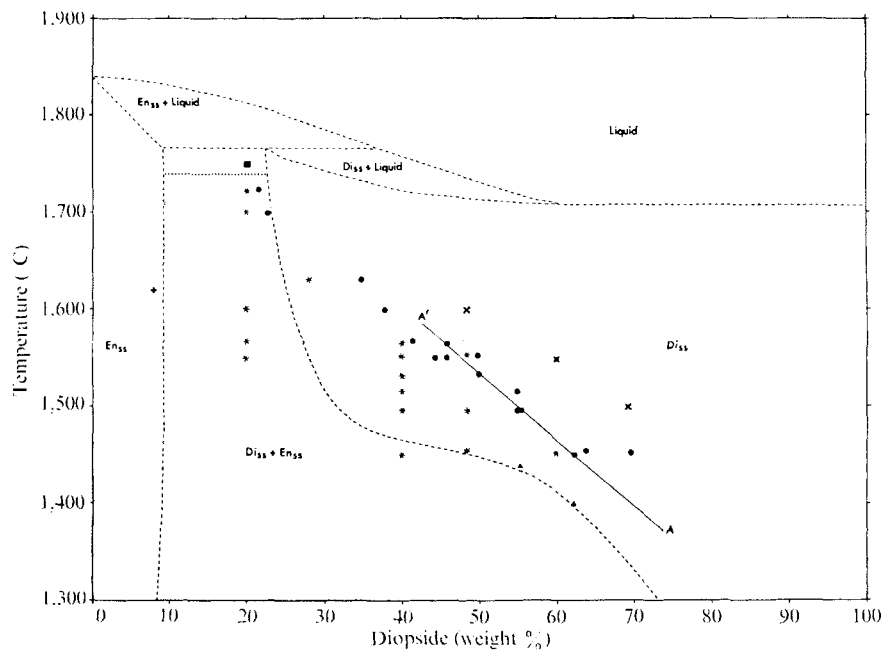


Fig. 1 Results obtained at 30 kbar from compositions lying along the diopside-enstatite join, shown in relation to the interpretation by Davis and Boyd³ (---). A-A' = The diopside limb of the diopside-enstatite solvus obtained from runs on the gel composition $\text{Di}_{40}\text{En}_{60}$ weight %. Results from gel starting materials: ★, bulk composition and temperature of runs which crystallised to a solid solution of diopside plus enstatite; ●, composition of diopside in equilibrium with enstatite, from X-ray measurements; ×, homogeneous diopside solid solution obtained; +, enstatite solid solution obtained; ■, enstatite solid solution plus liquid; ○, liquid; ▲, composition of diopside solid solutions coexisting with enstatite solid solution crystallised from the glass of bulk composition $\text{Di}_{40}\text{En}_{60}$ weight %; ·····, solidus in present work. SS, solid solution (in all figures).

products. When very fine-grained crystallised gels are used as starting material they react readily and completely to yield the equilibrium products.

A high pressure piston-cylinder apparatus (see ref. 5) was used in these experiments. Temperatures were measured using Pt/Pt 13 rhodium thermocouples with no correction for pressure effects. The starting materials were finely ground gels which had been recrystallised at 900 °C. Runs were made both on homogeneous gels ($\text{Di}_{40}\text{En}_{60}$, $\text{Di}_{50}\text{En}_{50}$) and on mechanical mixtures of pure diopside and enstatite gels (bulk compositions: $\text{Di}_{20}\text{En}_{80}$, $\text{En}_{28}\text{En}_{72}$, $\text{Di}_{40}\text{En}_{60}$, $\text{Di}_{60}\text{En}_{40}$, $\text{Di}_{80}\text{En}_{20}$). All compositions are quoted in weight %. A glass of composition $\text{Di}_{40}\text{En}_{60}$ was also used for more direct comparison with the earlier works^{3,4} which had used glass starting materials. All charges were dried at 1,050 °C for 1 h and sealed in platinum capsules. Runs were initially taken to 5 kbar above the desired pressure, the excess pressure being bled off when the required temperature was reached. In the temperature ranges 1,450–1,500 °C, 1,500–1,565 °C and above 1,600 °C, the durations of the runs were at least 4 h, 2 h and 0.5 h, respectively, except where stated. At 1,450 °C no change in the clinopyroxene composition occurred when experiments were run for longer

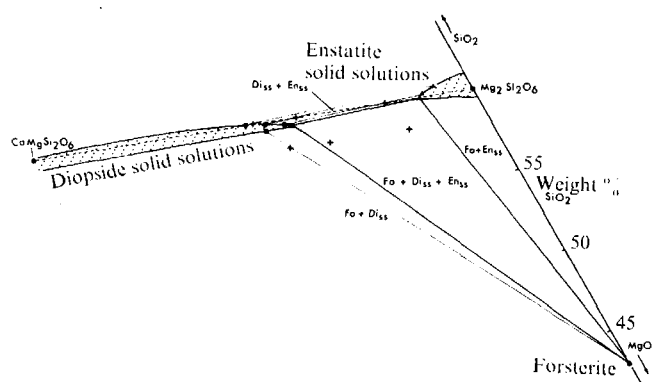
than 1.5 h, and it was therefore assumed that equilibrium had been attained in all experiments used for drawing up the diopside solvus.

Products were identified by optical and X-ray diffraction methods. Attempts made to analyse clinopyroxenes using the electron microprobe failed because of the small grain size (< 5 μm) of the crystals; it was not possible to focus the electron beam on a unique grain, and interference from adjacent crystals produced a wide range of clinopyroxene compositions (for example, $\text{Ca}/(\text{Ca} + \text{Mg}) = 0.10\text{--}0.25$ in one of the coarser grained charges). The X-ray diffraction technique used chromium radiation in an evacuated chamber. Compositions of clinopyroxenes were estimated from the relationship reported by Davis and Boyd³ which our study verified as valid between 20 and 69 weight % diopside. Clinopyroxene compositions could be obtained with a precision of $\pm 1\%$ diopside (99.7% confidence limits). The precision is rather less good for charges containing large amounts of enstatite. This technique is capable of resolving two clinopyroxenes differing by as little as 10 weight % of diopside. Clinopyroxenes which crystallised in equilibrium with enstatite at 30 kbar from the starting composition $\text{Di}_{40}\text{En}_{60}$ lay along the curve A-A' (Fig. 1) irrespective of whether or not the homogeneous gel or the mixed diopside-enstatite gel was used. Clinopyroxenes which crystallised from starting compositions $\text{Di}_{20}\text{En}_{80}$ and $\text{Di}_{28}\text{En}_{72}$ lay away from this curve by up to 4 weight % diopside towards enstatite; clinopyroxenes from starting compositions $\text{Di}_{50}\text{En}_{50}$ and $\text{Di}_{60}\text{En}_{40}$ lay up to 7 weight % diopside away from curve A-A' towards pure diopside. When 10 weight % of forsterite was added to each of the gels $\text{Di}_{20}\text{En}_{80}$ and $\text{Di}_{40}\text{En}_{60}$ at 1,550 °C and 1,565 °C, constant clinopyroxene compositions of $\text{Di}_{41.5}\text{En}_{58.5}$ and $\text{Di}_{42}\text{En}_{58}$, respectively, were generated from both compositions. This indicates that in detail the parageneses have the form shown in Fig. 2.

Experiments using glass of composition $\text{Di}_{40}\text{En}_{60}$ as starting material yielded homogeneous clinopyroxene above 1,440 °C as required by Davis and Boyd's³ phase diagram which was obtained from runs on glass charges. At 1,440 °C and 1,400 °C this starting material crystallised to enstatite and clinopyroxenes which lay exactly on the diopside limb of Davis and Boyd's³ solvus.

Runs on the homogeneous gel $\text{Di}_{40}\text{En}_{60}$ at 1,450 °C yielded enstatite and a clinopyroxene Di_{56} after 10 s and a progressively more magnesian clinopyroxene (up to Di_{50}) in longer runs of up to 30 min. This trend towards the solvus found by Davis

Fig. 2 Part of the system $\text{MgO-SiO}_2\text{-CaO}$ (weight %) at 30 kbar and 1,550 °C, showing bulk compositions of charges (+), and measured clinopyroxene compositions (●), in relation to inferred variation of SiO_2 in the diopside solid solution field (the extent of which along the join $\text{CaMgSi}_2\text{O}_6\text{-Mg}_2\text{SiO}_4$ is taken from refs 6 and 7). Tie lines to inferred enstatite compositions (▲) are shown intersecting the $\text{CaMgSi}_2\text{O}_6\text{-Mg}_2\text{SiO}_4$ join.



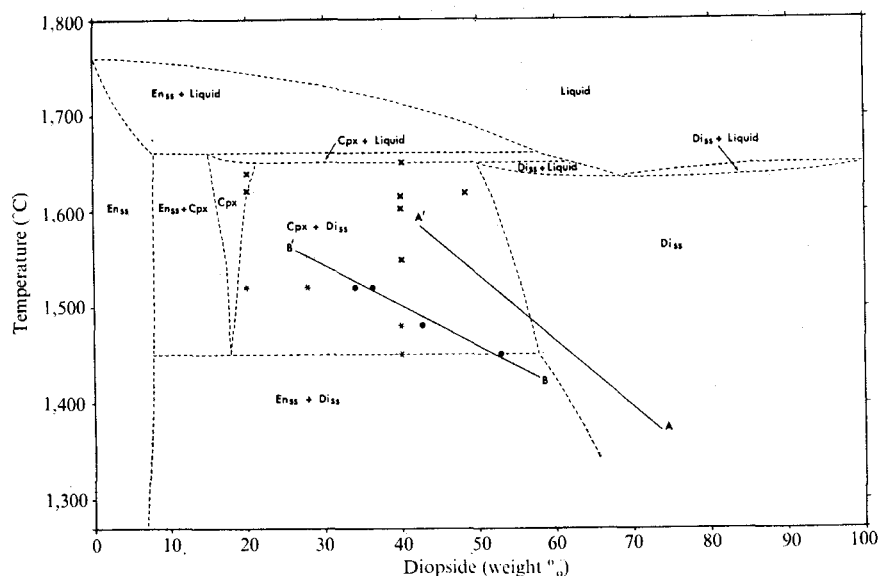


Fig. 3 Results obtained in this work at 20 kbar from compositions lying along the diopside-enstatite join, shown in relation to the interpretation by Kushiro⁴. B-B' = Approximate position of the diopside limb of the diopside-enstatite solvus obtained from experiments on gel starting materials. A-A', and all symbols as in Fig. Cpx, clinopyroxene.

and Boyd³ was reversed in longer runs, as the clinopyroxene changed towards the solvus A-A' (Di₆₀ after 50 min; Di₆₃ after 90 min), indicating the latter to represent the closer approach to equilibrium. The effects may indicate that the exchange of Ca and Mg between the two pyroxenes proceeds more rapidly than the adjustments in silica content implied in Fig. 2.

Figure 3 shows the results at 20 kbar from compositions lying on the diopside-enstatite join in relation to phase boundaries obtained⁴ using glass charges. We found that enstatite coexists with a diopside lying close to the curve B-B' (Fig. 3). The boundary B-B' at 20 kbar is significantly displaced towards En relative to A-A' at 30 kbar, but this pressure effect is for charges which were not saturated with forsterite. The pressure effect is, however, also significant in forsterite saturated charges. Forsterite (10 weight %) was added to the mixed gel of bulk composition Di₂₀En₈₀ at 1,520 °C. The clinopyroxene which crystallised in equilibrium with enstatite and forsterite had the composition Di_{37.5}En_{62.5}. When the temperature of the forsterite bearing assemblage at 30 kbar is raised by 30 °C the clinopyroxene becomes 4 weight % richer in diopside. The effect of pressure on the diopside solvus (20–30 kbar) at 1,535 ± 15 °C is to increase the amount of apparent diopside by 0.8 weight % per kbar in forsterite saturated assemblages. The effect in the stoichiometric join CaMgSi₂O₆–Mg₂Si₂O₆ is not necessarily the same as this. Translated into temperatures this would represent an error of +7 °C per kbar for assemblages equilibrated at a higher pressure than that of the experimental determinations.

Material of composition Di₂₀En₈₀ yielded a homogeneous clinopyroxene at 1,620 °C and diopside and enstatite at 1,520 °C. Kushiro⁴ predicted an assemblage of pigeonite plus minor diopside for the 1,520 °C run. Material of composition Di₄₀En₆₀ yielded diopside plus enstatite at 1,450 °C and 1,480 °C and a single homogeneous clinopyroxene of composition Di₄₀En₆₀ at 1,550 °C. The two higher temperature runs lie within the reported⁴ field of diopside plus pigeonite stability but fail to verify its existence. Runs on bulk composition Di₄₀En₆₀ at 1,620 °C using homogeneous gel and glass starting materials, and at 1,614 °C using the mixture of diopside and enstatite gels, all yielded a homogeneous clinopyroxene. There was again no evidence for the coexistence of two clinopyroxenes as required by Kushiro⁴. He reported⁴ that he was not able consistently to generate the pigeonite plus diopside assemblage. It is possible that the two clinopyroxene field is a quenching phenomenon. Deliberately slow quenching of the Di₄₀En₆₀ gel, run at 1,620 °C failed, however, to produce two clinopyroxenes,

nor were they obtained from glass or homogeneous gel starting materials of the Di₄₀En₆₀ composition when run at 1,620 °C in the same equipment used⁴ by Kushiro. We conclude that there is no stable field of pigeonite separate from a diopside field on this join at 20 kbar.

In the light of these results the temperatures on the geotherm estimated by Boyd³ may require some revision, and the method should only be applied to olivine saturated assemblages when compared with a diopside solvus determined in the presence of excess forsterite.

S. HOWELLS
M. J. O'HARA

Grant Institute of Geology,
University of Edinburgh,
Edinburgh EH9 3JW, UK

Received January 23; revised February 14, 1975.

- ¹ Nixon, P. H., and Boyd, F. R., in *Lesotho Kimberlites* (edit. by Nixon, P. H.), 48–56 (Lesotho National Development Corporation, Maseru, 1973).
- ² Boyd, F. R., *Geochim. cosmochim. Acta*, 37, 2533–2546 (1973).
- ³ Davis, B. T. C., and Boyd, F. R., *J. geophys. Res.*, 71, 3567–3576 (1966).
- ⁴ Kushiro, I., *Yb. Carnegie Instn Wash.*, 67, 80–83 (1968).
- ⁵ O'Hara, M. J., Richardson, S. W., and Wilson, G., *Contr. Miner. Petrol.*, 32, 48–68 (1971).
- ⁶ Davis, B. T. C., *Yb. Carnegie Instn Wash.*, 63, 165–171 (1964).
- ⁷ Kushiro, I., *ibid.*, 63, 101–108 (1964).

Crustal structures in central southern Africa

WE announce preliminary results from the geophysical reconnaissance of Botswana carried out during 1972 and 1973. Tertiary to Recent sands and sediments in the Kalahari Basin totally obscure the solid geology over 70% of the country, but these new results give exciting first indications of the disposition of geological features in the concealed 'basement' when related to the better known geology of the surrounding territories. The new data also suggest the extent of the concealed sedimentary and volcanic strata of Karroo age, and their possible relationship to the recent, if slight, tectonic instability in the heart of the continental plate. This work forms part of Botswana's contribution to the International Geodynamics Project¹.

A network of 2,131 gravity stations was established over Botswana's thinly populated 575,000 km² land area, over much of which access and communication by land is difficult and slow. A network of gravity base stations at 23 strategic airstrips was established using two LaCoste and Romberg gravimeters

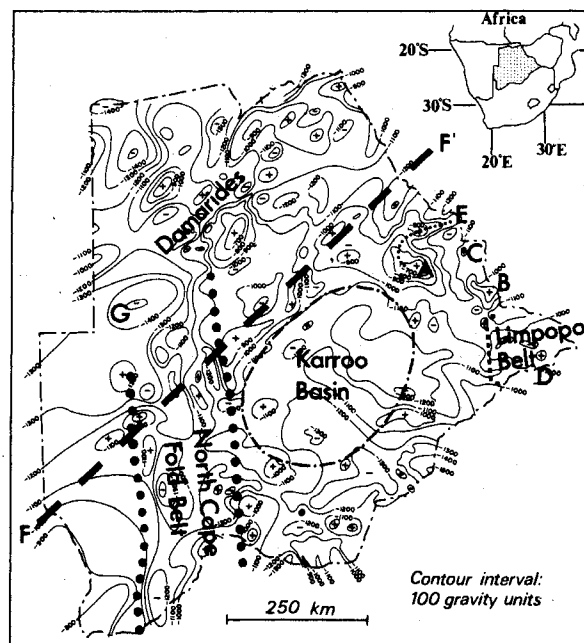
transported by light aircraft² and the intermediate stations were reached either by truck or helicopter. Altitude control of barometric observations was provided by the 1964 primary triangulation (Directorate of Overseas Surveys) and by more recently completed precise levelling routes (Department of Surveys and Lands, Botswana). Navigation equipment on trucks was essential for position fixing. Total-field magnetic observations were made at 1,300 of the gravity stations.

A mean negative Bouguer anomaly of about 1,150 gravity units (1 g.u. = 10^{-6} m s⁻² = 0.1 mgal) persists over the whole country, reflecting the isostasy of the monotonously flat continental plateau which stands at about 1,000 m above sea level, while locally Bouguer anomalies range in value from -700 g.u. to -1,500 g.u.

In the geologically better known areas in the east of the country the denser rocks of the isolated greenstone belts or "schist relics"³ in the Archaean basement produce local positive gravity anomalies. By far the largest of these is at Matsitama (A, Fig. 1) and smaller ones are found at Tati (B), Vumba (C), Baine's Drift (D) and Maitengwe (E). Similar anomalies over schist belts in both South Africa and Tanzania have been discussed by Darracott^{4,5}. But even the anomaly over the Barberton Mountainland, South Africa, is much smaller, both in area and amplitude, than that at Matsitama. Here causative dense rocks must extend westwards below the cover of Karroo and Kalahari sediments beyond the confines of the exposure mapped geologically and a further, arcuate, extension of the Matsitama anomaly (A-E) runs north, then north-eastwards to link up with the anomaly at Maitengwe. The cause of this arcuate structure is entirely concealed by more recent cover, but a connection between the two "schist relics" was predicted on purely geological grounds³. It is convincingly delineated by the new geophysical evidence which may therefore have considerable economic importance.

The gravity 'high' in the area of the northern Transvaal and southern Rhodesia border, recognised in earlier surveys and attributed to the Limpopo Mobile Belt, quickly disappears when traced westwards into Botswana, becoming extinct west of Selebi-Pikwe. This corroborates recent thinking⁶ that the Limpopo Belt may terminate in this region and does not continue westwards to intersect the Damaran Belt⁷ nor swing southwards to join the North Cape Fold Belt.

Fig. 1 Bouguer anomaly map of Botswana showing preliminary interpretation.



In east central Botswana a large featureless area of the Bouguer anomaly map seems to characterise the Karroo Basin and suggests its western and southern limits, where there is no geological control as yet⁸. An earlier study of the seismicity of Botswana⁹ shows this same area as notably aseismic. Together these factors tend to support Green's suggestion⁸ that the deposition of the Karroo strata was controlled by epeirogenic movements of a rigid continental plate, rather than by multiple or migratory rifting, at least in the area indicated in Fig. 1.

The geological significance of a "Kalahari Seismicity Axis"⁹, based on recent epicentre data and supported by the disposition of photogeological features, is well supported by the gravity data, where a considerable anomaly gradient follows much of the length of the axis (F-F'). Seismic activity may be associated with an abrupt change in crustal thickness or density along this line. This might be the edge of the foreland to the Damaran orogenic belt, an incipient Rift, or both, but whatever its significance, the Kalahari seismicity axis divides the gravity anomaly map into areas of distinctly different "texture". South-east of the seismicity axis the gravity anomaly field is largely featureless, devoid of steep gradients and localised anomalies, while to the north-west numerous large anomalies, both positive and negative, are evident. Many of the anomalies are elongated in an approximately NE-SW direction, giving an overall impression of a strong north-easterly strike over the entire north-west half of Botswana. This area includes the seismically active Okavango Delta where recent seismic refraction studies have shown¹⁰ that some of the faults determining the south-east margin of the delta⁹ are downthrown by as much as 300 m to the north-west and probably postdate the Karroo. But faulting even on this scale can only make a relatively small contribution to the observed gravity anomalies.

The only area of extensive rock outcrop in western Botswana is the Ghanzi Ridge where there is a major gravity "low" (G). Here the rocks are considered to be part of the Damara orogenic belt and, by extrapolation, we suggest that Damarides may underlie the entire area north-west of the Kalahari seismicity axis. Anomalous density distribution resulting from folding and intrusion could account for the pattern of gravity anomalies characterising this area. This, however, contrasts with the findings of Darracott¹¹ that the Mozambique orogenic belt is featureless in gravity anomalies compared with the adjacent cratonic area in East Africa. We do, however, believe that it is significant that both the Okavango seismicity and the 'Kalahari Seismicity Axis' seem broadly associated with areas we now presume to be underlain by the Damaran orogenic belt.

Striking north from the vicinity of Tshabong in southernmost Botswana a clear trend extends that recognised in the gravity survey of South Africa¹², characterised by the large positive and negative anomalies in the vicinity of Olifantshoek in the North Cape Fold Belt. On the Bouguer anomaly map of Botswana this trend may be traced as far north as Ghanzi, where it seems to become engulfed in the north-easterly, supposed Damaran, trend. A major negative anomaly in the regional magnetic contours south of Ghanzi is coincident with the northerly gravity trend. We conclude that the North Cape Fold Belt (10⁹ yr) recognised in the Cape Province continues in a northerly direction as far as Ghanzi where it becomes lost in the Damaran remobilisation (5 × 10⁸ yr), the whole being concealed below the Karroo and Kalahari cover.

We thank Dr J. V. Hepworth and Dr D. Masson Smith for assistance.

C. V. REEVES*
D. G. HUTCHINS

The Geological Survey,
Private Bag 14,
Lobatse, Botswana

Received January 13, 1975.

*Present address: Department of Earth Sciences, The University, Leeds LS2 9JT, UK.

¹ Report No. 3, Inter-Union Commission on Geodynamics, 160 Botswana, (1972).

² Reeves, C. V., and Carruthers, R. M., *Bull. Geodes.* (in the press).

- ³ Litherland, M., *Nature phys. Sci.*, **242**, 125 (1973).
⁴ Darracott, B. W., *Trans. Geol. Soc. S. Afr.*, **77** (2) (in the press).
⁵ Darracott, B. W., *Trans. Geol. Soc. S. Afr.*, **78** (1) (in the press).
⁶ Key, R. M., and Hutton, S. M., *The Western Extremity of the Limpopo Mobile Belt* (Botswana Geol. Survey, 1974).
⁷ Crockett, R. N., and Mason, R., *Econ. Geol.*, **63** (5), 532-40, (1968).
⁸ Green, D., *The Karroo System in Bechuanaland* (Bull. No. 2, Botswana Geol. Survey, 1966).
⁹ Reeves, C. V., *Nature*, **237**, 95 (1972).
¹⁰ Greenwood, P. G., and Carruthers, R. M., *Rept No. 15*, Inst. Geol. Sci., Geophys. Division (1973).
¹¹ Darracott, B. W., *Nature*, **240**, 403 (1972).
¹² Smit, P. J., Hales, A. L., and Gough, D. I., *Geol. Survey Handbook 3* (Government Printer, Pretoria, 1962).

Geochronology of eastern Newfoundland

WHOLE-ROCK Rb-Sr isochrons from granitoid rocks intruded into the easternmost part of the Central Mobile Belt and the Avalon Platform of Newfoundland indicate that the bulk of the plutonic activity took place during Devonian and Carboniferous times. Of the nine intrusions that were dated, only one, a Cambrian granite from the Avalon Platform, was significantly older.

The Appalachian System in Newfoundland can be divided into three geological provinces, each characterised by a distinct geological history¹⁻³: the Western Platform, the Central Mobile Belt and the Avalon Platform. Volcanic rocks of Early Palaeozoic age (the Central Mobile Belt) are sandwiched between two blocks of Precambrian basement and Palaeozoic platformal sedimentary rocks. A more refined subdivision may also be possible, with the island divided into eight zones (designated A to H), each characterised by a distinct Ordovician and/or pre-Ordovician history of deposition and folding⁴. The northern and western parts of the island have been studied in detail, and the geology of those areas has furnished some of the basic observational information used to synthesise models for the evolution of orogenic belts⁵ and the origin and emplacement of ophiolite complexes⁶. The central parts of the island have not been so well studied.

Granitoid rocks make up a significant proportion of the total volume of rocks exposed in Newfoundland and contribute (Map 1231A, Geological Survey of Canada) about 40 per cent of the total area of granitoid rocks exposed in the Canadian Appalachians. The ages of a few of the intrusions are known from stratigraphic evidence, and some K-Ar measurements have been made on others, but the timing of the magmatic activity remains obscure. These K-Ar ages are mostly single determinations on single mineral phases, and must be interpreted with caution. The isotopic ages obtained so far range from 330 to 450 Myr, with the bulk of them between 340 and 400 Myr. These granites have been related to the Devonian Acadian Orogeny and the K-Ar ages seemed grossly in agreement with this opinion. Other intrusions, however, are believed to be significantly older⁷.

Here we summarise results of a geochronological study of granitoid rocks from Zones F, G and H in eastern Newfoundland. Full details will be published elsewhere. Nine intrusions were dated by the Rb-Sr whole-rock method (Fig. 1).

Rb-Sr ratios and concentrations were determined by X-ray fluorescence. The ratios and the concentrations are considered to be accurate to $\pm 3\%$ (95% confidence level) of the quoted value. Sr isotope ratios were measured on a 15-cm, 90°, solid-source mass spectrometer. Our reproducibility is one part in 700 at the 99% confidence level. Measurements of the Eimer and Amend Sr carbonate standard gave an $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7077 ± 0.0003 .

The whole-rock Rb-Sr ages (Table 1) are calculated on the basis of the $1.47 \times 10^{-11} \text{yr}^{-1}$ decay constant for ^{87}Rb . They indicate that: (1) Of the nine granites sampled, six are either Silurian or Devonian according to the 'absolute' time scale,

and thus could fall within the generally accepted time span associated with the Acadian Orogeny. (2) Bearing in mind the uncertainties assigned to each age, it seems that in Zones F and G, granite magmatism had started by the Silurian and continued into the early Carboniferous. Plutonic activity on the Avalon Platform (Zone H) extended much further back, at least into Cambrian times. (3) None of the granites in Zones F and G that were analysed are of pre-Middle Ordovician age. This is surprising because many of the foliated leucogranites, particularly those associated with the metamorphic terrain, were considered to be that old on geological grounds⁷.

Two models have been suggested for the geological evolution of eastern Newfoundland. Kennedy and McGonigal⁷ divided the region into three distinct terrains: a basement or gneissic terrain of possibly Precambrian age, a metasedimentary terrain of pre-Middle Ordovician age, and a Middle Ordovician sedimentary and volcanic terrain. Kennedy and McGonigal suggest that the gneissic rocks not only underlie the eastern part of the Central Mobile Belt but that they may also form a sialic basement to the Avalon Platform. This relationship between the underlying basement and the Gander Lake Group (redefined to include only the metasedimentary terrain) resembles that of the Precambrian (in part Grenville) rocks of the Western Platform and the overlying Cambro-Ordovician sequences. A similar relationship also exists between the basement gneisses of the western flank of the Central Mobile Belt and the overlying Fleur de Lys Supergroup. A correlation of the newly-defined Gander Lake Group and the Fleur de Lys Supergroup is considered in terms of two sedimentary units that form continental prisms thickening from the eastern and western platforms on to the central belt.

By contrast, the plate tectonic model of Strong *et al.*⁸ involves a south-easterly-dipping subduction zone, with the Avalon Platform forming a continental margin, and with the plutonic rocks of the Gander Lake Metamorphic Belt (Zone G) inter-

Fig. 1 Sketch map (after Strong *et al.*¹⁰) of the granitoid rocks of eastern Newfoundland. The numbered granites are: 1, Mt Peyton; 2, Middle Ridge; 3, Ackley City; 4, Freshwater Bay; 5, Middle Brook; 6, Cape Freels; 7, Terra Nova; 8, Swift Current; 9, St Lawrence.

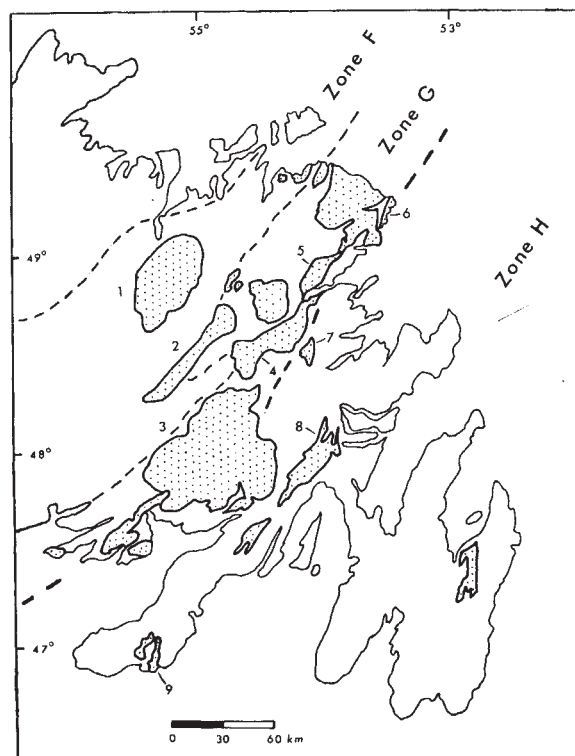


Table 1 The analytical data

Locality	Normative composition ¹⁰	Petrographic notes ¹⁰	No. of points	Age (Myr)	Mean square of weighted deviates (MSWD) [†]
St Lawrence	Granite	Alaskite granite-rhyolite porphyry-abundant veins and fracture fillings of fluorite	4	315 ± 10*	3.2
Terra Nova	Granite	Coarse-grained granite—also microcline, megacrystic variety	4	335 ± 20	0.3
Ackley City	Granite-quartz monzonite	Orange to red, coarse-grained porphyritic biotite granite, irregular masses of alaskite and aplite	4	345 ± 10	1.8
Freshwater Bay	Granite	Megacrystic microcline granite, in parts schistose-foliation trending NE-SW. Inclusions of metasediment and migmatite	4	360 ± 15	0.9
Middle Ridge	Granite	Pink medium-grained and central garnetiferous leucocratic phase	4	370 ± 10	3.8
Mt Peyton	Granite-granodiorite	Syenite, granite, diorite, monzonite	4	375 ± 15	12.7
Cape Freels	Granite	To east, homogeneous megacrystic granite—to west, augen gneiss	4	400 ± 15	0.3
Middle Brook	Granite-granodiorite	Microcline megacrystic granite—metamorphic texture in parts, augen gneisses	6	435 ± 35	3.1
Swift Current	Granite-diorite	Pink equigranular medium-grained granite, also contaminated variety with granitised volcanic and sedimentary rocks	4	510 ± 20	7.7

*95% confidence level.

†This quantity gives some idea of the goodness of fit of the points to a straight line, and in the ideal case approaches a value of one if the points fit the line within experimental error⁸. In practice, the MSWD is strongly dependent on the precise estimation of the analytical uncertainties, and on the total number of points used to define the isochron, so that for our results any MSWD less than about five probably represents a suitable fit of the points to a straight line. Only the results from Mt Peyton show any real sign of a deviation from 'ideal' behaviour.

puted as Palaeozoic intrusions above the subduction zone. The metamorphic belt running along the eastern part of the Central Mobile Belt is related to the same subduction zone, and reflects metamorphic activity above a down-going plate sometime during the Palaeozoic. The direction of subduction from north-west to south-east is supported by some gross geochemical trends (in particular a definite eastward increase in the average K content of the granitoid plutons of eastern Newfoundland) and by a zonation of many Newfoundland mineral deposits.

To distinguish unequivocally between either of these two models by using both the geological information and the data in Table 1 is difficult, but the Rb-Sr ages provide certain important constraints. Perhaps the most important finding is that most of the granites that were sampled are 400 Myr old or younger. The prediction that several of the foliated granites intruded into the gneissic terrain may be of either Precambrian or pre-Middle Ordovician age is difficult to reconcile with the new data even though the higher mean square weighted deviates (MSWDs) associated with some plutons may indicate that the Rb-Sr system was disturbed sometime after intrusion. The intense foliation associated with at least two of the megacrystic granites, the Cape Freels and the Freshwater Bay, suggests a much younger (post-400 Myr) period of intense deformation and/or metamorphism. On the other hand, a variation in age across an ocean-arc and continent system, which would be intuitively expected from a simple model of subduction as suggested by Strong *et al.*, is not at all apparent from our data, although any igneous phenomena associated with a downgoing plate are not going to be straightforward in terms of either age or spatial distribution.

We thank A. R. Berger, D. F. Strong and H. Williams for discussions and D. F. Strong for samples. T. J. S. Cole and D. P. Menagh were responsible for the mass spectrometer, and J. S. Stevenson and P. F. DeWolf provided technical assistance. This work was supported in part by the NRC and the President's Fund of Carleton University.

KEITH BELL
JOHN BLENKINSOP

Department of Geology,
Carleton University,
Ottawa, Canada

Received December 9, 1974.

¹ Kay, M., *Bull. Am. Assoc. petrol. Geol.*, **51**, 579-600 (1967).

² Williams, H., *Am. J. Sci.*, **262**, 1137-1158 (1964).

³ Kay, M., and Colbert, E. H., *Stratigraphy and Life History* (Wiley, New York, 1965).

⁴ Williams, H., Kennedy, M. J., and Neale, E. R. W., *Geol. Assoc. Canada, Spec. Paper 11*, 181-261 (1972).

⁵ Bird, J. M., and Dewey, J. F., *Bull. geol. Soc. Am.*, **81**, 1031-1060 (1970).

⁶ Dewey, J. F., and Bird, J. M., *J. geophys. Res.*, **76**, 3179-3207 (1971).

⁷ Kennedy, M. J., and McGonigal, M. H., *Can. J. Earth Sci.*, **9**, 452-459 (1972).

⁸ Brooks, C., Hart, S. R., and Wendt, I., *Revs. Geophys. Space Phys.*, **10**, 551-577 (1972).

⁹ Strong, D. F., Dickson, W. L., O'Driscoll, C. F., Kean, B. F., and Stevens, R. K., *Nature*, **248**, 37-39 (1974).

¹⁰ Strong, D. F., Dickson, W. L., O'Driscoll, C. F., and Kean, B. F., *Rept 74-3* (Newfoundland Dept Mines and Energy, 1974).

Origin of gasoline range alkanes in the deep sea

It is believed that C₄ to C₇ hydrocarbons in petroleum are formed by the cracking of organic matter at depths generally exceeding 1,000 m at temperatures in excess of 50 °C (refs 1-3). Also, none of the alkanes in the butane-heptane range are formed biologically as far as is known at present. Consequently, it is thought that they do not occur in shallow, Recent sediments. In 1962, I analysed 22 samples of Recent sediments from 7 different environments and verified that these hydrocarbons were not present at the p.p.m. level⁴ although traces of a few hydrocarbons such as butane, isobutane, isopentane and *n*-heptane have been found⁵⁻⁸. No identification of individual hexanes or heptanes has been reported except when there has been clear evidence of seepage from deeper source sediments⁹.

Using more sensitive techniques I have detected the widespread occurrence of practically the entire suite of C₄-C₇ alkanes of petroleum at the ng g⁻¹ level in shallow, low temperature marine sediments. The yields generally correlate with organic carbon. This suggests that diagenetic reactions which form traces of C₄-C₇ hydrocarbons are occurring much earlier and at much lower temperatures than most geochemists had expected.

The analytical technique¹⁰ consists of placing frozen core material in a metal container completely filled with water and then introducing a known volume of helium to form a gas cap. After thawing, the container is placed in a boiling water bath, then agitated and a sample of gas is withdrawn and analysed on a gas chromatograph fitted with a 200 foot, 0.01 inch internal diameter hexadecene-hexadecane-Kel F capillary column. Each peak was identified against a known standard.

Table 1 Total yield of C₄–C₇ alkanes from Deep Sea Drilling Project (DSDP)

Location*	DSDP Core no.*	Interval (cm)	Age	Sediment depth (m)	Lithology	Organic carbon (weight %)	C ₄ –C ₇ alkanes (parts per 10 ⁹)†
1. Gulf of Mexico 23°N 92°W	1-3D-1-2	70–150	Upper Pleistocene	28	Silty clay	0.81	18
2. Off California 39°N 127°W	5-34-4-4	86–126	Lower Pliocene	113	Clay mud	0.56	5.5
3. Off California 39°N 127°W	5-34-5-4	0–40	Lower Pliocene	120	Clay mud	0.47	28
4. Bengal Basin 9°N 91°E	22-217-16-4	10–30	Palaeocene	416	Nannofossil chalk	0.04	0.47
5. Bengal Basin 9°N 86°E	22-218-27-2	0–10	Middle Miocene	769	Clayey silt	0.14	6.1
6. Gulf of Aden 14°N 52°E	24-233A-7-4	0–20	Lower Pliocene	229	Nanno-ooze	2.95	109
7. Indian Ocean 33°S 39°E	26-250A-10-6	6–26	Lower Pliocene	414	Silty clay	0.20	0.46
8. Indian Ocean 31°S 88°E	26-254-25-6	120–140	Miocene	228	Clay mudstone	0.10	0.88
9. South of Tasmania 49°S 147°E	29-280A-6-2	125–150	Upper Oligocene	109	Diatom silt	0.36	18
10. South of Tasmania 49°S 147°E	29-280A-19-0	0–50	Lower Eocene	443	Silty clay	0.83	1,284
11. South of Tasmania 49°S 147°E	29-280A-21-1	130–150	Lower Eocene	511	Silty clay	0.61	6,386
12. West of Tasmania 42°S 143°E	29-282-4-0	33–55	Upper Miocene	28	Nanno-ooze, clay	0.21	32
13. West of Tasmania 42°S 143°E	29-282-18-1	90–100	Lower Eocene	295	Clayey silt	4.8	1,964
14. Sea of Japan 39°N 138°E	31-299-22-1	0–110	Pleistocene–Pliocene	200	Clay	0.95	22

* Exact well locations and core descriptions are published in the Leg Reports of the DSDP, US Government Printing Office, Washington, DC. DSDP core numbers indicate in order: leg-hole-core-section.

† Parts per 10⁹ by weight, or ng per gram of dry sediment.

About 5% of the samples analysed so far, mostly from abyssal plain areas, have shown no detectable hydrocarbons even though the method is sensitive to 5 parts per 10¹² in a 100 g sample. This indicates there is no continuous source of contamination at the levels studied.

Table 1 shows the yield of C₄–C₇ alkanes from 14 deep sea samples. The samples range in age from Pleistocene to Palaeocene and in sediment depth from 28 to 769 m. Organic carbon contents vary from 0.04 to 4.8%. Nannofossil chalk (Table 1, no. 4) had the lowest yield, and oozes and clays from the outer continental margins (Table 1, nos 1–3, 6, 9–13) had the highest yields. The yield of C₄–C₇ alkanes generally increased with increasing organic carbon content except in the vicinity of Tasmania where very high yields in the range of 1,000–6,000 parts per 10⁹ were obtained. These high yields are associated with a basalt intrusion which occurs at a depth of about 520 m in Hole 280A. Previous studies^{11,12} have shown that the high temperatures caused by igneous intrusions can generate large quantities of hydrocarbons from the organic matter in adjacent shales. Distribution of these hydrocarbons can be erratic because of variations in migration pathways, but yields are invariably higher than average. The three deepest samples near Tasmania have a mean yield of 3,211 µg alkanes per gram of carbon, which compare with a mean yield of 22 µg alkanes per gram of carbon for the rest of the samples. This suggests that some of the alkanes in the deep Tasmanian samples have migrated from sediments heated by the intrusion.

When factors such as temperature are relatively constant, a correlation between hydrocarbon yields and the total organic carbon content of the sediment indicates that the alkanes are autochthonous although there is no way to prove conclusively that this is so. When the yield of C₄–C₇ alkanes is plotted against organic carbon, excluding the three deep Tasmanian samples, the correlation coefficient is +0.75. This indicates that those alkanes are autochthonous. There is apparently no correlation with lithology, age or depth. The deepest sample in any one hole generally has the highest yield. Marked increases in yield, however, occur over very short distances, as in the first two samples of Hole 34. These differences cannot be a

result of the temperature increase with depth. The highest temperature to which these samples have been exposed is estimated to be about 45 °C for the sample from the deepest part of the Bengal Basin. Most samples have never been above 25 °C. More likely, differences in yield, as in Hole 34, are related to variability in the type of organic matter. A few samples were treated with HF and HCl to remove the mineral matter, and the organic residues were examined microscopically. Organic matter in the deep sea samples was generally amorphous whereas the clays from basins along continental margins contained woody fragments, spores and pollen. The Indian Ocean sample from Hole 254, which is over 1,500 km from any continent, showed a surprising amount of woody material. Palynological studies¹³ showed, however, that these sediments were deposited in a shallow lagoon during Miocene times.

Individual hydrocarbon yields for eight representative samples are shown in Table 2. Results to date indicate that certain hydrocarbons form very early during diagenesis, whereas others form very late. The earliest formed hydrocarbons are generally the butanes, pentanes, 2- and 3-methylpentanes and methylhexanes, *n*-hexane, *n*-heptane, and methylcyclohexane. The hydrocarbons that seem to form latest are 2,2-, 2,3- and 2,4-dimethylpentanes; 1,*cis*-3 and 1-*trans*-2-dimethylcyclopentanes, and 3-ethylpentane. About 1 sample in 20 contains a trace of 2,2,3-trimethylbutane. A few samples contain olefins which are now being examined using mass spectrometry. Large anomalies in individual hydrocarbon yields are scattered erratically through the samples. For example, *n*-pentane constitutes 43% of sample 7 and 42% of sample 6. Sample 13 contains 31% 2-methylhexane. When it is realised that an individual hydrocarbon represents only 0.0001% of the organic matter, it is understandable that such anomalies can be attributed to a very small part of the organic matrix. For example, sample 13 may contain a few easily reducible compounds with 2-methylhexane chains from the original plant material. It can be expected that such anomalies would disappear when the organic matter was buried to sufficient depth to generate large quantities of a variety of hydrocarbons by thermocatalytic reactions.

Table 2 Individual C₄-C₇ alkane yields (ng per gram of sediment)

	7*	8	9	1	14	12	6	13
Isobutane	0.082	0.033	0.34	0.25	1.82	0.29	2.5	8.7
n-Butane	0.021	0.040	1.63	1.36	2.61	1.49	1.2	25.6
Isopentane	Abs	0.31	0.54	0.54	0.21	0.68	1.3	32.5
n-Pentane	0.20	0.21	4.64	6.55	0.42	4.41	45.8	93.6
2, 2-Dimethylbutane	Abs	Abs	0.65	0.17	0.64	0.60	0.5	12.4
Cyclopentane	Abs	Abs	0.30	0.17	0.29	0.45	3.3	185
2, 3-Dimethylbutane	0.092	0.12	0.23	Abs	0.17	0.41	Abs	30.0
2-Methylpentane	Abs	Abs	0.59	2.54	1.74	1.19	3.0	43.3
3-Methylpentane	0.026	Abs	3.17	0.71	0.61	0.98	1.8	21.2
n-Hexane	0.009	0.07	0.68	1.33	1.24	1.24	6.9	36.5
Methylcyclopentane	Abs	0.04	0.38	0.50	0.64	2.36	2.6	18.1
2, 2-Dimethylpentane	Abs	Abs	Abs	Abs	Abs	Abs	Abs	28.4
2, 4-Dimethylpentane	Abs	Abs	Abs	Abs	0.56	Abs	Abs	14.1
Cyclohexane	Abs	Abs	0.03	0.30	0.99	1.18	3.0	35.3
3, 3-Dimethylpentane	Abs	Abs	Abs	0.12	0.78	0.56	0.6	9.3
1, 1-Dimethylcyclopentane	Abs	Abs	Abs	0.12	0.76	0.42	0.6	35.3
2-Methylhexane	0.02	Abs	0.65	0.4	1.56	1.39	3.6	618
2, 3-Dimethylpentane	Abs	Abs	Abs	0.16	Abs	1.22	Abs	Abs
1, cis-3-Dimethylcyclopentane	Abs	Abs	Abs	0.22	Abs	Abs	Abs	62.4
3-Methylhexane	Abs	0.05	0.86	0.18	1.30	2.01	5.7	35.7
1 trans-3-Dimethylcyclopentane	Abs	Abs	Abs	0.66	0.31	2.80	Abs	63.0
1 trans-2-Dimethylcyclopentane	Abs	Abs	Abs	Abs	0.78	Abs	4.8	118
3-Ethylpentane	Abs	Abs	Abs	Abs	0.67	Abs	Abs	12.1
n-Heptane	0.005	0.005	1.40	0.62	1.09	2.53	9.8	58.8
1, cis-2-Dimethylcyclopentane	Abs	Abs	Abs	0.22	0.39	1.56	1.1	34.5
Methylcyclohexane	Abs	Abs	1.61	0.84	2.14	4.63	10.5	332
Total	0.46	0.88	18	18	22	32	109	1,964

* Sample numbers as in Table 1.
Abs=Absent

Hydrocarbon ratios even at this early stage of generation are similar to those in crude oil for some, but not all, hydrocarbon pairs. For example, the ratio of 2- to 3-methylpentane in crude oil averages 1.7 (ref. 14). The mean ratio in the samples of Table 2 is 1.9. The ratio of 1-trans-2- to 1-cis-2-dimethylcyclopentane is about 2.7 in crude oils and 3.3 in these samples. Between methylcyclohexane and cyclohexane the ratio is 2.5 in crude oil and 4.4 in these samples, excluding sample 9 which has almost no cyclohexane. An inverse relationship is noted in the ratio between 2 and 3-methylhexane; it is 0.8 in crude oils and 1.1 in these samples, excluding the anomalous sample 13.

All studies to date indicate in general that some hydrocarbons form earlier than others. When yields at specific locations are compared, however, it seems that some organic matter forms a wider spectrum of hydrocarbons more readily than others. Thus, the Pleistocene sample from a depth of 28 m in the Gulf of Mexico contains 5 more hydrocarbons in the C₄-C₇ range than the Oligocene sample from a depth of 109 m south of Tasmania, even though both samples have a total of 18 ng of hydrocarbon per gram of sediment.

The occurrence of these gasoline range hydrocarbons in shallow marine sediments may be an early indicator of the capability of sediments to generate petroleum. On the other hand, a significant point of this study is that time and the particular nature of the organic matter are not alone sufficient to ensure the generation of commercial quantities of petroleum hydrocarbons. A higher temperature is required than the 15-45 °C range recorded here. For example, the Eocene clays associated with the Lake Maracaibo oilfields in Venezuela have about 1 mg of C₄-C₇ alkanes per gram of carbon at about 75 °C compared with the 18 µg per gram of carbon in the shallow Eocene samples of this study⁴. In the Jurassic sediments of the Paris Basin, there is⁵ about a 100-fold increase in the C₄-C₇ alkanes in going from sediments at about 35 °C to those at about 100 °C.

I thank the Scaife Family Trusts and the National Science Foundation for support of this project.

JOHN M. HUNT

Woods Hole Oceanographic Institution,
Woods Hole,
Massachusetts 02543

Received January 10, 1975.

- 1 Cordell, R. J., *Bull. Am. Ass. Petrol. Geol.*, **56**, 2029 (1972).
- 2 Dow, W. G., *Bull. Am. Ass. Petrol. Geol.*, **58**, 1253 (1974).
- 3 Tissot, B., et al., *Bull. Am. Ass. Petrol. Geol.*, **55**, 2177 (1971).
- 4 Duntson, M. L., and Hunt, J. M., *Bull. Am. Ass. Petrol. Geol.*, **46**, 2246 (1962).
- 5 Sokolov, V. A., *Gostoptekhnizdat*, May, 59 (1957).
- 6 Veber, V. A., and Turkeltaub, N. M., *Geologiya Nefti Gaza*, **8**, 39 (1958).
- 7 Erdman, J. G., et al., *134th Meet. Am. chem. Soc.*, Chicago (1958).
- 8 Emery, K. O., and Hoggan, D., *Bull. Am. Ass. Petrol. Geol.*, **42**, 2174 (1958).
- 9 McIver, R. D., in Ryan, W. B., et al., *Initial Rep. Deep Sea Drilling Project*, **XIII**, 813 (1973).
- 10 Hunt, J. M., in von der Borch, C. C., et al., *ibid.*, **XXII**, 673 (1974).
- 11 Hunt, J. M., *Proc. Second Int. Scient. Oil Conf., Budapest*, **2**, 394 (1962).
- 12 Baker, D. R., and Claypool, G. E., *Bull. Am. Ass. Petrol. Geol.*, **54**, 456 (1970).
- 13 Thierstein, H. R., *Initial Rep. Deep Sea Drilling Project*, **XXVI**, 632 (1974).
- 14 Smith, H. M., *Bureau of Mines Bulletin*, 642 (1968).

Acoustic observations of suspended particulate matter in the ocean

CLOUDS of suspended particulate matter in the ocean have been observed by satellite¹, sensed by nephelometers², and sampled by water bottles. We present here evidence that it may be possible to observe oceanic suspended particulate matter acoustically. The particulate matter discussed is thought to have arisen from a dredging operation. It is also thought that the suspended matter is present at lower concentrations than any in any other case recorded acoustically³. Certain questions, as yet unresolved, regarding acoustic scattering strengths are also discussed.

An experiment to determine the feasibility of acoustic surveys of suspended sediments was conceived during field testing of a 20-kHz LODAR echo-sounding system. The LODAR system has a downward-looking acoustic beam of 12° by 18° and a pulse duration of 2.2 ms. Initial acoustic records of a cloud-like feature were coincident with visual sightings of suspended sediment down-current of a working dredge. During this initial experiment, the dredge was operating for five days out of eight and data were taken on both incoming and outgoing tides. In conjunction with the LODAR records, measurements were made with expendable bathythermographs (XBTs) and a continuous recording light beam transmissometer (LBT).

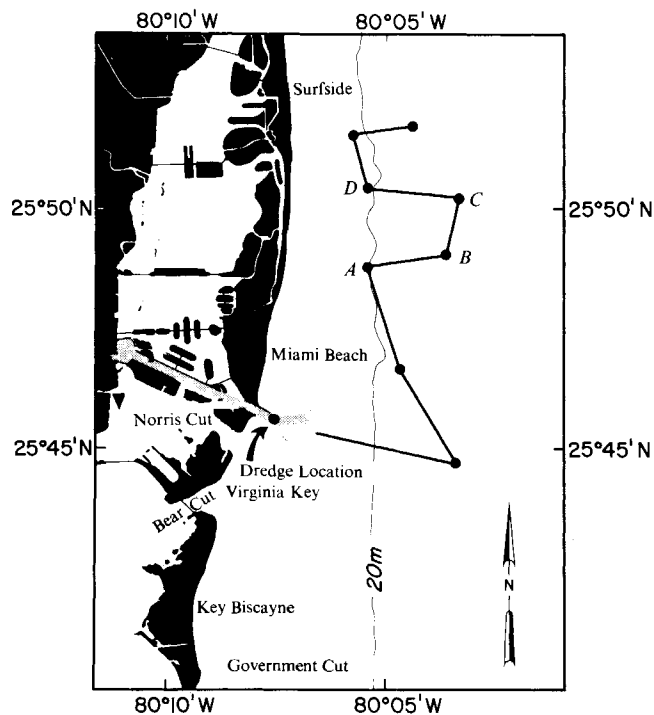


Fig. 1 Typical trackline in area of investigation (May 22, 1974, RV Virginia Key). A-D indicates locations shown on Fig. 2.

On the five days when the hydraulic suction dredge was working, a visible cloud of suspended sediment and the acoustic 'cloud' were observed down-current of the dredge. On incoming tides the sediment flowed into the bay where the water is too shallow to operate the LODAR system. On outgoing tides the suspended sediment flowed out of the channel; the surface manifestation disappeared between 200 and 600 m seaward of the channel, depending on the state of the sea. Figure 1 is a typical trackline and Fig. 2 is the acoustic record from part of that track. The same general trackline was traversed each day. The minimum volume of the cloud estimated from acoustic records was about 60,000 m³. During the three days when the dredge was not in operation, clouds were not observed either by the acoustic equipment or by visual observation of any surface manifestation.

The continuous recording LBT records the percentage transmission of a beam of white light 2 cm in diameter over a 1-m path; filters maximise the sensitivity at 475 nm. Although the light transmission characteristics involve more than one parameter^{3,4}, the instrument is useful for obtaining approximate particulate concentrations and distributions. LBT station depths were limited by the length of telemetering cable aboard,

but shallow casts (less than 100 m) indicated a reduction in transmittance of between 10% and 15% in the upper regions of the cloud. The developed cloud occupied a region between inflection points on the XBT temperature traces (Fig. 3). A volumetric concentration on the order of 0.01–1%, obtained from the acoustic data, enabled us to carry out a first-cut check of acoustic and transmissometer readings. For the LBT used⁵, a reduction in transmittance from 85% to 60% implies a particulate concentration of roughly 0.03%, so that the acoustic and transmissometer devices are consistent.

The observed acoustic 'cloud' cannot be accounted for either by temperature anomalies or by a package of contaminated bay water which would occur independently of dredging operations (see ref. 6). Temperature anomalies, however, seem to influence the location in depth of the acoustic cloud which occupies a region between inflection points on the XBT records (Fig. 3). This observation is consistent with the finding that temperature-induced density gradients are an important controlling factor in the transport of fine-grained sediment⁷. Large biological reflectors cannot account for the cloud as there is no evidence of point source reflectors on the records. The possible role of small biota in producing such a cloud requires further investigation. Although unlikely, the stirring up of bottom nutrients by the dredging operation may have produced a short-lived bloom of microscopic biota.

It is unlikely that the cloud results from microbubbles produced by organic processes in the sediment and released by the dredging operation. An extraordinary amount of decay of organic matter would have to occur to produce clouds of the magnitude observed on every outgoing tide when the dredge was in operation. Such extraordinary quantities would have been observed as seeps emanating from the bottom when the dredge was inoperative.

The observed behaviour and properties of the acoustic cloud support our interpretation that it is the product of acoustic scattering from suspended sediment particles in the water column. The nature of a suspended sediment cloud and its movements would be affected by the tides, temperature-induced density gradients, and the northward flow of the Gulf Stream current. The observed cloud varied in size, shape, and location corresponding to the changes in dredging operations and tides. The tides affect both the direction of flow in Government Cut and the flow of warmer bay waters out to sea on outgoing tides, thus inducing the density gradients at the outflow of the cut. Also, the observed acoustic clouds moved to the north on leaving the cut, with the easternmost extent of the cloud approaching the Gulf Stream.

How is it, though, that particles with diameters that may be of the order of 1–100 μm can reflect detectable sound signals at 20 kHz? The present hypothesis is that the sediment cloud may have regions of relatively high particulate concentration which are detectable but not resolvable using the acoustic system described here. Studies are under way to determine the validity of that hypothesis.

Theoretical work and further field work are under way to

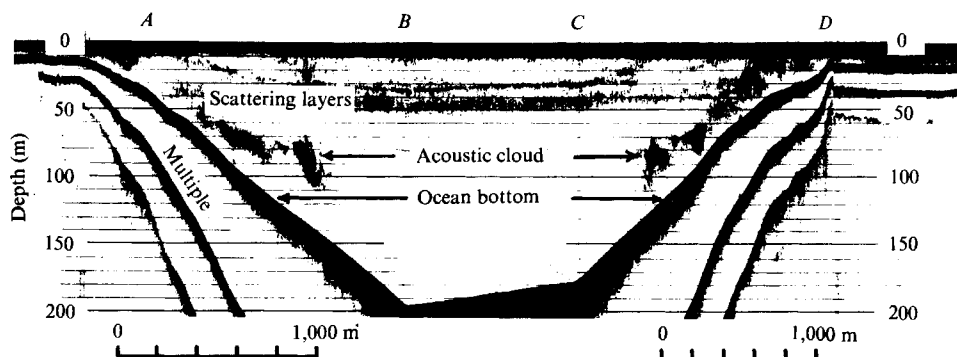


Fig. 2 Acoustic LODAR record along typical trackline, locations as indicated on Fig. 1. The shallow scattering layer is indicated. May 22, 1974.

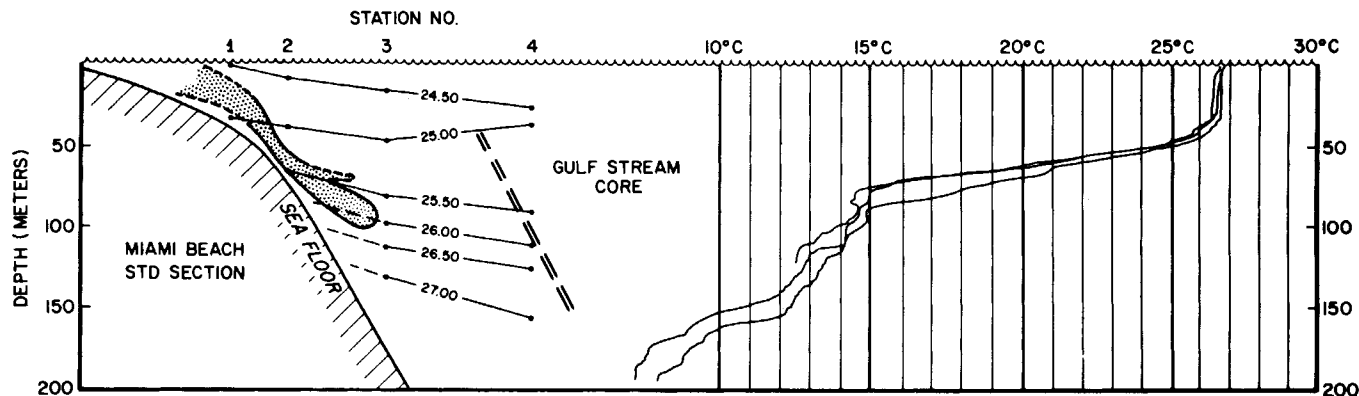


Fig. 3 Sketch of the acoustic cloud with overlay of Sigma- T (σ_T) curves (T. Lee and D. Mayer of National Oceanic and Atmospheric Administration) with XBTs on same scale, May 22, 1974. $\sigma_T \equiv (\rho - 1) \times 1,000$ where ρ = water density. σ_T is obtainable from STD (salinity-temperature-depth) measurements. Gulf Stream core based on salinity of March 27, 1974.

develop what may prove to be a valuable survey tool, with immediate applications for the sedimentologist, geologist, ecologist and pollution monitoring agencies.

Dr David Drake assisted with the transmissometer measurements; his comments and advice are appreciated. We thank the referee who pointed out the consistency check between the acoustic level received and the transmissivity decrease.

J. R. PRONI
D. C. RONA
C. A. LAUTER JR
R. L. SELLERS

Atlantic Oceanographic and Meteorological Laboratories,
Environmental Research Laboratories,
National Oceanic and Atmospheric Administration,
Miami, Florida 33149

Received December 11, 1974; revised February 25, 1975.

- ¹ Klemas, V., Borchardt, J. F., and Treasure, W. M., *Remote Sensing of Environment*, 2 (4) (1973).
- ² Ewing, M., and Thorndike, E. M., *Science*, 147, 1291-1294 (1965).
- ³ Gallenne, B., *Estuar. coast. mar. Sci.*, 2, 261-272 (1974).
- ⁴ Jerlov, N. G., *Optical Oceanography* (Elsevier, Amsterdam, 1968).
- ⁵ Beardsley, G. F., Pak, H., Carder, K., and Lundgren, B., *J. geophys. Res.*, 75, 2837-2845 (1970).
- ⁶ Proni, J. R., and Apel, J. R., *J. geophys. Res.* (in the press).
- ⁷ Drake, D. E., *Shelf Sediment Transport* (edit. by Swift, D. J. P., Duane, D. B., and Pilkey, O. H.), 307-331 (Dowden, Hutchinson, and Ross, Stroudsburg, 1972).

Vertebrate localities in the Karroo System of the Luangwa Valley, Zambia

PROLIFIC vertebrate localities within the Karroo System of the northern part of the Luangwa Valley in Zambia have been known since Dixey's investigations of 1928 and 1935¹. They were the subject of extensive collecting in 1960-61 by the then Geological Survey of Northern Rhodesia², and again in 1963 by a joint British Museum (Natural History)-University of London expedition³. Two fossiliferous horizons are recognised in the Luangwa Valley, the Madumabisa Mudstones corresponding to the Upper Permian *Daptocephalus*-zone of South Africa, and the Ntawere Formation which is regarded as a little older than the Manda Formation of Tanzania and corresponds approximately to the Beaufort-Stormberg boundary of the Karroo System. In 1972 members of the Geological Survey of Zambia investigated the middle Luangwa Valley in and around the game reserves, discovering two new, potentially important localities. The first lies in the Munyamadzi Corridor between the northern and southern game reserves and is an exposure of the Madumabisa Mudstones yielding abundant therapsid and some pareiasaur remains. The fossils

are, however, preserved in calcareous mudstone nodules which lack the extensive, hard ferruginous covering which makes the specimens from the Upper Luangwa so difficult to prepare. The second is an exposure of the Ntawere Formation in the northern game reserve and yielded archosaur-like teeth along with the bivalve *Unio karrooensis*. As a consequence of these discoveries members of the Oxford University Museum were invited to participate in an expedition during the summer of 1974, to collect fossil vertebrates from the series of new localities in the middle Luangwa Valley.

North of the Munyamadzi River extends a nearly flat alluvial plain, beyond which the terrain rises about 70 m in 6-7 km. This hilly belt is dissected extensively by stream beds and eight localities were found within the 'donga' type erosion system associated with these streams. Most of the localities lay just below a remnant of horizontal grit or sandstone on an interfluvium and consisted of small gullies cutting through the soil and soft rock, which often coalesce to form wide, shallow, bare hollows. The fossiliferous rock is a near-horizontal, brown and grey, fine-grained calcareous mudstone, with frequent nodules that are of the same rock type but are more calcareous, and in which the fossils themselves occur. Large amounts of bone have weathered out and lie strewn over the surface but numerous good specimens were found still *in situ*.

A basically similar locality was found on the Musina River some 18 km further north, apparently in the same geological horizon. The specimens collected from these Madumabisa Mudstones were characteristic of the *Daptocephalus*-zone of South Africa. They consisted of abundant dicynodont skulls often with postcranial bones associated, about a dozen gorgonopsid skulls including one with much of the postcranial skeleton, two whaitsiid-like theropodians, a complete procynosuchid skeleton resembling *Parathrinaxodon proops*⁴, a pareiasaur skull and partial postcranial skeleton and, at one particular locality, numerous remains of fishes, including hybodont-like fin spines, palaeoniscid scales and the partial skull of an *Acrolepis*-like palaeoniscid. All these specimens have since proved very suitable for the acetic acid method of preparation.

Two localities along the Lokokwa River in the northern game reserve were also investigated. They seem to be exposures of the Ntawere Formation and consist of a sequence of mottled red-brown and greenish mudstones and siltstones with some thin, light grey, flaggy sandstones and lenses of intraformational conglomerate of calcareous pellets. Vertebrates were, however, extremely scarce and the only specimen of likely value is a piece of the conglomerate containing part of a small archosaur skeleton. Otherwise only a few surface fragments of labyrinthodont and dicynodont nature, and a large archosaur tooth were found, along with abundant *Unio karrooensis* specimens.

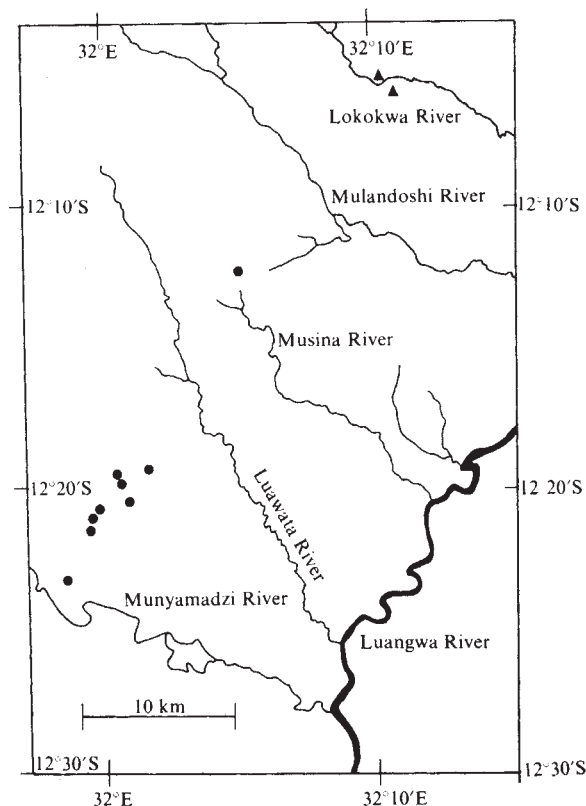


Fig. 1 Karroo localities of the middle Luangwa Valley. ●, Madumabisa Mudstones; ▲, Ntawere Formation.

The collection is now in the Oxford University Museum where it is under preparation. I express my gratitude to the staff of the Geological Survey of Zambia, particularly Dr Alan Drysdall for the initial invitation and Mr and Mrs Colin Kerr, for their cooperation throughout the expedition. I also thank the Wildlife Department and the Monuments Commission of Zambia for the necessary permits, and the Royal Society for a grant. Mr H. P. Powell of the Oxford University Museum and Mr C. D. Kerr of the Geological Survey of Zambia accompanied me during this expedition.

T. S. KEMP

University Museum,
Oxford OX1 3PW, UK

Received January 13, 1975.

¹ Dixey, F., *Rep. geol. Surv. Nyasaland*, 24 (1936).

² Drysdall, A. R., and Kitching, J. W., *Northern Rhodesia Ministry of Labour and Mines. Memoir of the Geological Survey No. 1* (1963).

³ Attridge, J., Ball, H. W., Charig, A. J., and Cox, C. B., *Nature*, 201, 445-449 (1964).

⁴ Parrington, F. R., *Phil. Trans. R. Soc.*, B226, 121-142 (1936).

Do children draw men with arms coming out of the head?

CHILDREN'S drawings raise formidable taxonomic problems. Consider the two extremely common drawings in Fig. 1. There are good reasons for selecting the 'conventional man' as a standard against which to assess the 'tadpole man' but we do not know how to do it. It may be that the tadpole man is lacking a trunk¹⁻⁴ or that it has the trunk amalgamated with the head^{4,5} (and at least two other descriptions are also plausible). In practice, authors have tended to opt for the following subset of possible hypotheses: (1) the trunk is omitted and the arms attached according to a rule 'attach arms to head'; (2) the trunk is omitted and the arms drawn on the head by default even though the child has a rule specifying

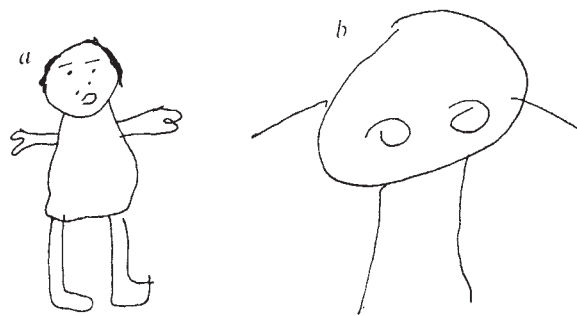
the trunk as a locus of insertion for limbs; (3) the trunk is not differentiated from the head, and therefore the rule 'attach arms to trunk' is not violated. The present experiment was designed to test the plausibility of such accounts. Children were given incomplete figures and asked to draw limbs. The results show that the tendency to attach arms to the head or the trunk can be brought under lawful control in such a way as to discriminate against the above hypotheses in favour of one based on the concept of a 'production error'.

As in any binary-choice experiment, the primary goal is to find a set of stimuli which will reliably affect the relative strengths of the two responses under examination, namely, attaching arms to the head or to the trunk, at the same time as satisfying the condition that the stimuli occur at approximately equal intervals on an underlying scale. Such a set is series *a* in Fig. 2, in which each stimulus pair sums to the same overall height, with the central pair being of equal size to give a point of objective and subjective equality (as established in a pilot study). From a classical psychophysical standpoint, series *b* is experimentally somewhat neater since it is based on the method of constant stimuli, in which a fixed 'standard' is paired with varying 'comparison' stimuli. In the present case, one of each pair of circles is held constant, at a diameter of 3 cm, with the other circle varying on an equal interval scale, although this allows for covariance in the overall height. Both series were used in the present experiment. Similarity of the results obtained from the two series will therefore produce confidence in the assumptions involved in the scaling procedures adopted.

Children (140) in local playgroups aged between 2, 3, and 4 yr (mean = 3.8, s.d. = 0.7) were each asked to draw a man on A4 paper. They were then individually asked to complete a drawing consisting of a head. Then each was presented with a booklet containing a random ordering of one of the series shown in Fig. 2 and asked to complete each drawing before turning the page to the next. The locus of insertion of the arms was noted, and the results conditionalised on the spontaneous drawings by dividing subjects up into groups according to whether they spontaneously drew conventional men, tadpole men, or scribbles.

Fifty-four subjects drew a conventional man (group M), 33 drew a tadpole man (group T) and 35 produced scribbles (group S). The remainder, who largely produced disjointed conventional men, were ignored for present purposes. Analysis of variance showed that group M were reliably older than groups T and S (mean = 4.0, 3.7, and 3.4 yr, respectively) at the 5% level of significance. Groups M and T tended to be consistent when given the predrawn head in that 48 out of 54 of group M turned it into a conventional man and 28 out of 33 of group T turned it into a tadpole. Interestingly, none of group S scribbled, and 20 of them produced a tadpole man, whereas 15 produced a conventional man. When given the series of incomplete drawings, every child put the legs on the trunk. The results for arm attachment is shown in Table 1. Clearly group M remained relatively consistent in attaching arms to the trunk. The results for groups T and S seem to resemble one another. Calculation of Cochran's *Q* confirms

Fig. 1 Drawings of a man (a) conventionally, (b) as a tadpole figure, by two preschool children.



the unequal distribution of locus of attachment across the conditions, and linear contrast on proportions⁶ is significant for group T (95% confidence limits being 3.68–1.68 for series *a*, 3.56–0.36 for series *b*). For group S, the linear trend just fails to reach acceptable significance (95% confidence limits being 2.00 to –0.12 for series *a*, 3.64 to –0.28 for series *b*). Thus there is clear evidence that for the individuals in group T, the larger the head in relation to the trunk the greater tendency to draw arms on the head. Thus group T can attach arms to the trunk but equally can be led to ignore the trunk. The same may well be true of group S, but the evidence is equivocal.

The results for group T are evidence against the operation of the stable rules 'attach arms to head' or 'attach arms to trunk' which hypotheses (1) and (2) promote. With regard to hypothesis (3), the results show that a trunk-attachment rule may be violated. In other words, any putative rule operates only in interaction with the particular graphic configuration. Therefore, rather than regarding the drawing as a direct expression of the child's conceptual knowledge⁷, we should consider what kind of performance factor could result in the

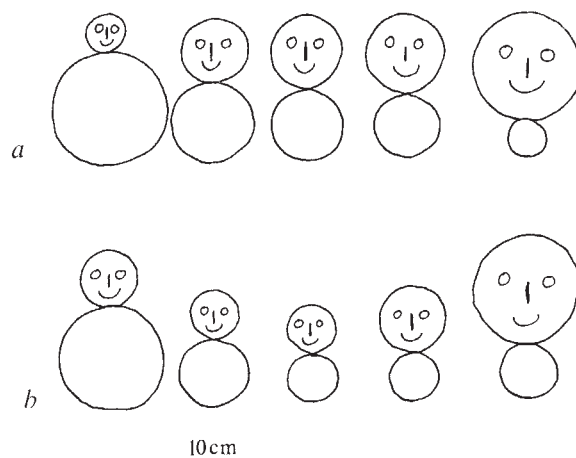


Fig. 2 Freehand incomplete drawings presented for completion. Series *a* constructed by holding overall size constant, series *b* by varying size around a constant component.

Table 1 Proportion of each group of subjects who attached arms to the head rather than the trunk of an incomplete figure, as a function of head-trunk ratio

		Group M (<i>n</i> = 27)	Group T (<i>n</i> = 17)	Group S (<i>n</i> = 13)
Series <i>a</i>	Head-trunk ratios			
	0.1	0.00	0.06	0.15
	0.7	0.00	0.06	0.15
	1.0	0.00	0.18	0.15
	1.5	0.07	0.41	0.31
	8.5	0.11	0.76	0.54
Series <i>b</i>	Head-trunk ratios			
	0.3	0.00	0.00	0.12
	0.6	0.04	0.20	0.12
	1.0	0.07	0.27	0.06
	1.7	0.07	0.53	0.44
	3.8	0.19	0.73	0.81

observed lawful variation. We suggest that a useful concept is 'production error', namely a characteristic defect in an executive routine, according to the following argument.

The child has the task of constructing a complex spatial array by bringing into relationship graphical elements which can only be produced in serial order. Typically children and adults talk about, and draw, a man in rather a fixed sequence: head, trunk, arms, legs. Published surveys on drawings^{8–11} suggest that if a drawing is incomplete, it tends to be the trunk and arms which are partially drawn or omitted. Note that the trunk is the second element in the pair head-trunk. With regard to the limbs, it was observed in the present experiment when group T produced a spontaneous tadpole man that 80% of the sample drew the limbs in the order legs-arms ($\chi^2 = 5.12$, 1 d.f., $P < 0.05$), that is, the opposite way round to conventional description. Thus for this group of subjects, we propose that their production sequence would typically be head-trunk, legs-arms, and that irregularities are prone to occur in the second element of each pair, that is, the children are liable to sample from the first element in each category, resulting initially in a head plus legs. This means that the child produces a centralised drawing in which the pencil is brought back to a central configuration rather than continuing on a vertical path. (It is this process that the incomplete drawings elicit.) This analysis accords both with Kellogg's¹² classification of drawings (though she overinterprets centralisation as radial symmetry) and with recent evidence¹³ showing that for young children, local configurational constraints may preclude them from effectively planning vertical orientation in human figure drawing. The reason why legs are the preferred limbs may be attributable to an early bio-mechanical bias towards vertical strokes, revealed in free scribbling¹⁴, since in simple drawings

such "postural propensities are powerful enough to assert themselves"¹⁵.

Thus we suggest that the tadpole man is associated with production problems in programming spatial layout rather than with any peculiar conceptual scheme. Note that group S, the scribblers, were also prone to head-attachment of the arms. In following up some individual scribblers we have found that they are prone to this variability for some months before they produce a recognisable conventional man or tadpole (in one case for over a year). This suggests that such drawing phenomena¹⁶ may be indices of general production problems, rather than being simply specific solutions which arise through repeated 'near misses' in drawing a man. The general problems may well be ones of serial-order programming. In this case, the common practice of scoring a single human-figure drawing may be unsuitable as a method of assessing conceptual intelligence in children of the age range studied here, in that the face validity of the draw-a-man test is challengeable and the completion task permits abilities to be shown by scribblers which they cannot show simply on the demand "draw a man".

N. H. FREEMAN

Department of Psychology,
8–10 Berkeley Square,
Bristol, UK

Received October 31, 1974; revised February 13, 1975.

- Lowenfeld, V., and Brittain, W. L., *Creative and Mental Growth* (Macmillan, New York, 1964).
- Dileo, J. H., *Young Children and their Drawings* (Brunner, New York, 1970).
- Machover, K., *Personality Projection in the Drawing of the Human Figure* (Thomas, Springfield, 1949).
- Luquet, G. H., *J. Psychol. norm. Path.*, **17**, 684–710 (1920).
- Arnheim, L., *Art and Visual Perception* (Faber, London, 1957).
- Marasciulo, L. A., and McSweeney, M., *Psych. Bull.*, **67**, 401–412 (1967).
- Goodenough, F. L., *Measurement of Intelligence by Drawing* (Harcourt, New York, 1926).
- Snyder, R. T., and Gaston, D. S., *J. clin. Psychol.*, **26**, 377–383 (1970).
- Hurluck, E. B., and Thompson, J. L., *Child Dev.*, **5**, 127–138 (1934).
- Ames, L. B., and Ilg, F. L., *Genet. Psychol. Monogr.*, **68**, 247–307 (1963).
- Shapiro, T., and Stine, J., *Psychoanal. Study Child.*, **20**, 298–309 (1965).
- Kellogg, R., *Analysing Children's Art* (National, Palo Alto, 1970).
- Goodnow, J. J., and Friedman, S., *Dev. Psychol.*, **7**, 10–16 (1972).
- Connolly, K., and Elliott, J., in *Ethological Studies of Child Behaviour* (edit. by Blurton-Jones, N.), 329–383 (Cambridge University Press, 1972).
- Gesell, A., and Ames, L. B., *J. genet. Psychol.*, **68**, 45–61 (1946).
- Freeman, N. H., *Perception*, **1**, 123–140 (1972).

Discrimination between parasitised and unparasitised hosts in the parasitic wasp *Pseudeucoila bochei*: a matter of learning

THE female of the parasitic wasp *Pseudeucoila bochei* (Hymenoptera: Cynipidae) lays her eggs in larvae of different *Drosophila* species, and only one parasite emerges from each host. The parasite is able to discriminate between parasitised

and unparasitised host larvae¹, but this ability does not completely prevent super-parasitisation. On investigating the probable causes of super-parasitisation, we discovered that the female wasp has to learn to discriminate, learning being that process which manifests itself by adaptive changes in individual behaviour as a result of experience².

Six possible causes were analysed, two of which proved to play an important role in the explanation of super-parasitisation (see Table 1). One of these—which must be related to the

Table 1 Possible causes of super-parasitisation by *Pseudeucoila*

Cause	Importance
1. A female lays more than one egg at an oviposition	—
2. A female does not recognise hosts parasitised by another female	—
3. A female lays a second egg after the first oviposition within the period which is needed for building up the factor which causes avoidance of super-parasitisation	±
4. Two or more females lay eggs simultaneously in one host	±
5. A female's tendency to oviposit increases when she encounters only parasitised hosts for a long period; she will lay eggs in these	+
6. A female has not yet learnt to discriminate	+

—, Never found.

±, Not important.

+, Important.

variation in the tendency of the wasp to oviposit—will be discussed in more detail elsewhere. Here, we report that in certain conditions this tendency may be strong and thus cause a high degree of super-parasitisation. This may happen when the parasite-host ratio is very high and/or when the time during which the hosts are exposed to the parasite(s) is very long.

The other cause, that the wasp has not yet learnt to discriminate, would very easily lead to a high degree of super-parasitisation. During experiments, parasitised larvae were offered to parasites which had never laid eggs before. The inexperienced parasites accepted these unsuitable host larvae (Table 2) as easily as unparasitised hosts (Table 3). The total number of contacts with, and rejections of, the parasitised larvae (Table 2) was tested against the total number of contacts with, and rejections of, the unparasitised larvae (Table 3): $\chi^2 = 1.4$, $P > 0.05$. Note that the general frequency of contacts was lower in the tests with parasitised larvae. The explanation of this phenomenon will be published elsewhere.

Inexperienced wasps may parasitise many already parasitised hosts in succession and thus waste a large number of their eggs. The parasites did refuse some of the larvae, although this also occurred in the control experiment, in which only unparasitised hosts were presented (Table 3).

If a batch of unparasitised larvae is presented first, and a batch of parasitised hosts thereafter, the wasps will almost completely avoid ovipositing in the latter (Table 4:

Table 2 Number of contacts with and rejections of hosts by inexperienced wasps during three or five tests of 30 min

Female	No. of tests	Total no. of contacts	Total no. of rejections
1	3	43	1
2	3	33	6
3	5	75	11
4	5	58	9
5	5	66	0
Total	21	275	27

Host larvae containing one parasite egg were offered in each test. The average number of contacts and rejections per test was about the same. After an oviposition the host was removed and replaced by a new, once-parasitised host.

Table 3 Number of contacts with and rejections of hosts by inexperienced wasps during five tests of 30 min

Female	No. of tests	Total no. of contacts	Total no. of rejections
1	5	98	5
2	5	72	4
3	5	142	6
4	5	95	21
5	5	110	1
Total	25	517	37

Unparasitised host larvae were offered in each test. The average number of contacts and rejections per test was about the same. After an oviposition the host was removed and replaced by a new, unparasitised host.

$\chi^2 = 47.3$, $P < 0.005$). The age of the wasps has no influence on their ability to discriminate; the only factor that matters is whether or not they had oviposited in an unparasitised host. From then on the parasites are able to distinguish parasitised from unparasitised hosts. These experiments show that the wasps have to learn to discriminate between parasitised and unparasitised larvae.

We discovered another phenomenon, which is probably related to the former. The inexperienced wasps may learn to discriminate, even when they encounter none but parasitised larvae containing for example one or two eggs. In this case they distinguish between hosts with one egg and hosts with two eggs, and avoid oviposition in the latter. As soon as the wasps encounter mainly hosts containing two eggs they leave the site.

We have shown previously¹ that the wasps not only discriminated between parasitised and unparasitised hosts, but also distinguished hosts with different numbers of eggs. At that time the functional significance of this ability remained rather obscure. In view of our present findings, however, learning may

Table 4 Number of contacts with and rejections of hosts by inexperienced wasps during 30 min of observation

Female	Test no. 1 Unparasitised		Test no. 2 Parasitised	
	No. of contacts	No. of rejections	No. of contacts	No. of rejections
1	8	0	38	36
2	23	1	34	34
3	16	0	31	30
4	14	0	24	24
Total	61	1	127	124

Unparasitised host larvae and once-parasitised host larvae were offered in succession. After an oviposition the host was removed and was replaced by an unparasitised host (test number 1) or a parasitised host (test number 2).

be seen as an important adaptation: if inexperienced wasps arrive at sites in which only parasitised larvae containing one egg are present, they do not continue laying (and thus waste all their eggs!), but leave the site when they have gradually encountered more and more larvae with two eggs. If, as we assume, at each oviposition some 'marking' substance is injected into the host (or is produced by the host), the wasp seems to be able to distinguish different concentrations of this substance, and not just notice its presence or absence.

We have presented¹ some mathematical models on the distribution of parasite eggs over the hosts. These models were based on a large number of egg distributions found in experiments. The majority of these experiments, however, were made with inexperienced wasps. We found substantial super-parasitisation in quite a number of cases, one of the causes of which may be that the inexperienced wasps used in the experiments did not start to parasitise at the same time. Since in each vial more than one wasp was present, wasps that started to parasitise later would have a better chance of meeting, as their first hosts, already parasitised larvae. These larvae

would most probably have been parasitised again by these inexperienced females.

Clearly, the larger the number of inexperienced wasps present in each vial, and the larger the differences in starting time of parasitisation between them, the higher the degree of super-parasitisation will be.

At that time we assumed that super-parasitisation was caused mainly by a strong tendency to lay eggs. In view of these new findings it seems that this would certainly apply to experienced wasps but that most of the super-parasitisation by inexperienced females should be attributed to the fact that they had not yet learnt to discriminate.

J. C. VAN LENTEREN
K. BAKKER

Department of Ecology, Zoological Laboratory,
University of Leiden, The Netherlands

Received January 20, 1975.

¹ Bakker, K., Eijssackers, H. J. P., van Lenteren, J. C., and Meelis, E., *Oecologia (Berl.)*, 10, 29–57 (1972).

² Thorpe, W. H., *Learning and Instinct in Animals* (Methuen, London, 1956).

Evolution and distribution of left-handed and planispiral coiling in snails

MOST extant snails (Gastropoda) are characterised by dextral (right-handed) coiling of the shell. Sinistral (left-handed) and planispiral (bilaterally symmetrical) coiling are uncommon among gastropods, particularly in the sea¹. The preponderance of one type of asymmetry over another contrasts with the approximately equal numbers of right-handed and left-handed stocks of conispirally coiled fossil nautiloid and ammonoid cephalopods^{2,3}, and the roughly equal occurrence of inequivalve bivalves in which either the left or the right valve is larger⁴.

There is no evidence that right-handedness is functionally superior or inferior to left-handedness. Here I attempt to explain previously unrecognised anomalies in the ecological and geological distribution of left-handed and planispiral coiling in gastropods by specifying some conditions of reduced stabilising selection under which the mechanisms giving rise to these coiling types could operate. My discussion is restricted to shells of adult snails. Limpet-like forms with bilateral symmetry as well as exceptional instances of sinistral coiling in otherwise dextral species are not considered.

Planispiral coiling is the presumed primitive condition in gastropods^{5,6}, but it has also been derived secondarily from dextral or sinistral forms in at least nine stocks of archaeogastropods (all pre-Tertiary marine), five or six stocks of freshwater and marine mesogastropods, one line of terrestrial Neritogastropoda, one line each of marine Thecosomata and Entomotaeniata, more than two lines of freshwater Basommatophora and eight planispiral stocks of terrestrial stylommatophorans (Table 1). No planispiral neogastropod is known.

Large size (2–10 cm in diameter) among planispiral shells is attained only among certain Recent land and freshwater forms (Corillidae, Systrophidae, Ariophantidae, Planorbidae, Pilidae) and among pre-Tertiary marine gastropods. All Recent marine gastropods with planispiral shells are less than 10 mm in diameter.

Topographically sinistral coiling may arise in any of three ways: First from a primitively planispiral ancestor; second by hyperstrophy from a dextral form, usually involving a planispiral intermediate phase, or third, by reversed symmetry from a dextral form. The developmental process of torsion, involving delayed appearance of the left compared with the right larval retractor muscle^{8,9}, predisposed primitive gastropods to dextral rather than sinistral coiling¹⁰. Hyperstrophic shells are characterised by an apex which is sunken below the apical edge of subsequently formed whorls, and by a base which protrudes as a false spire. Thus, while the shell is externally sinistral, the soft parts within are still dextral in organisation. Reversed symmetry

occurs when both shell and soft parts change in direction of coiling.

Excluding some slightly asymmetrical Bellerophonacea, very conservative estimates suggest that topographically sinistral coiling has independently arisen seven times among archaeogastropods (all but one lineage pre-Tertiary), four times among mesogastropods, six times among the marine neogastropods, twice in the Basommatophora (in one case leading to a major radiation of freshwater forms), and nine times among the land Stylommatophora (Table 1). An additional lineage contains primitive euthyneurans with sinistral larval shells together with the Thecosomata which retain sinistrality as adults. Within several lines of primitively sinistral freshwater Planorbidae, hyperstrophy has led to the evolution of pseudodextral coiling^{13,14}.

A necessary condition for the attainment of hyperstrophy appears to be the presence in the ancestral stock of an umbilicus, a cavity in the base of the shell created by the incomplete overlapping of adjacent whorls. Nearly all planispiral shells, most archaeogastropods, and most freshwater and land snails, are umbilicate; while most neogastropods and higher mesogastropods are not. It may be argued^{15,16} that incomplete whorl overlap renders a shell more fragile than a similarly shaped non-umbilicate shell of equal thickness. In the sea, all but the smallest snails are potentially exposed to vigorous water movements and, especially in the tropics, to shell-breaking predators¹⁷. Small snails can often occur in the boundary layer at the water–substrate interface, where water movements are diminished, or live among algae or in other habitats where shell-breaking predators can find prey less effectively^{1,17}. Hyperstrophy, involving a phylogenetic lowering of the true spire concomitant with an increase in umbilical width, should thus be rare among larger Recent marine gastropods because of an overall reduction in shell strength; but incipient hyperstrophy leading to a planispiral condition might still arise in small-shelled stocks.

In pre-Cretaceous seas, selection among large snails for strong shells may have been less intense than it is today since crabs and teleost fishes, which are among the most effective shell-breaking predators on Recent marine gastropods, were rare¹⁸ or absent¹⁹ before the Cretaceous. Data based on compilations from Knight *et al.*²⁰ suggest that about 74% of conispiral genera in the largely Palaeozoic Pleurotomariacea (Archaeogastropoda) have umbilicate shells, while calculations based on all known genera of Trochacea (Triassic to Recent)²⁰ and on all Recent North American genera²¹ of this archaeogastropod superfamily yield estimates of 60% and 58% umbilicate genera respectively. The mostly non-umbilicate neogastropods and higher mesogastropods became important elements in the fauna only in the Mesozoic and especially the Cainozoic. Thus secondarily planispiral and hyperstrophic stocks could have evolved in pre-Tertiary times even among large-shelled gastropods.

The shells of many freshwater and land snails are fragile compared with those of marine forms. Many freshwater snails (notably Basommatophora) are annuals, and may adapt to predation by maintaining high reproductive rates rather than by developing impenetrable shells. This may also be true among many land snails, which in addition need not contend with water waves or currents. As in pre-Tertiary marine snails, selection against umbilicate shells is likely to be much reduced among freshwater and land snails, and hyperstrophy is more likely than among large Recent marine forms.

The taxonomic distribution of reversed symmetry may be explained partly by considering methods of larval dispersal. All 18–19 Recent lineages of gastropods with true sinistral coiling, except the Triphoridae and Thecosomata, develop without a planktonic stage. Since reversed symmetry becomes evident at cleavage¹⁰, and as most mutations affecting events at such early developmental stages are associated with harmful side effects, the maintenance in a population of a mutant gene for reversed symmetry is unlikely except possibly in some small

Table 1 Ecological and geological distribution of planispiral (p), hyperstrophic (h) and sinistral (s) gastropods

Classification	p, h, and s taxa	Habitat	Age	Classification	p, h, and s taxa	Habitat	Age
Subclass Prosobranchia				Conacea			
Order Archaeogastropoda				Conidae	s <i>Conus adversarius</i>	Mar. b.	Mio.-Plio.
Bellerophonacea	p Whole superfamily	Mar. b.	Cam.-Recent	Terebridae	s <i>Terebra inversa</i>	Mar. b.	Plio.-Quat.
Macluritacea*				Turridae	s <i>Antiplanes</i>	Mar. b.	Recent
Macluritidae	p <i>Macluritella</i> , s? <i>Omphaloceras</i>	Mar. b.	Cam.-Tri.	Subclass Euthyneura			
Euomphalacea*				Order Entomotaeniata			
Euomphalidae	p <i>Lesueurella</i> , <i>Amphiscapha</i> , <i>Planotectus</i>	Mar. b.	Cam.-Dev.	Pyramidellidae	p <i>Cyclostremella</i> ?	Mar. b.	Perm.?-Recent
Weeksiidae	p Whole family	Mar. b.	E. Ord.	Order Thecosomata			
Helicotomidae	p <i>Ophileta</i>	Mar. b.	Dev.	Limacinidae	h Most Limacinidae	Mar. pl.	Tert.-Recent
Pleurotomariacea				Limacinae	p <i>Limacina inflata</i>	Mar. pl.	Recent
Gosseletiniidae	p Gosseletiniinae	Mar. b.	Ord.-Perm.	Peraclididae	h Whole family	Mar. pl.	Recent
Porcellidae	p Whole family	Mar. pl.?	Cret.	Order Basommatophora			
Eotomariidae	s? Agnesiinae	Mar. b.	Dev.-Jur.	Ellobiacea			
Platyceratacea				Ellobiidae	h? <i>Blauteria</i> ¹²	Mar. b.	Recent
Holopeidae	s? <i>Antitrochus</i>	Mar. b.	Dev.	Ancylacea	s Physidae, many Planorbidae and Bulinidae	Fr.	Tert.-Recent
Amberlyacea					p Many Planorbidae and Bulinidae	Fr.	Tert.-Recent
Cirridae	s? Whole family	Mar. b.	Tri.-Jur.		h Many Planorbidae ^{13,14}	Fr.	Tert.-Recent
Clisospiracea				Order Stylommatophora			
Clisospiridae	s? Clisospirinae	Mar. b.	Ord.-Sil.	Achatinellacea	s Many Achatinellidae and Partulidae	Land	Cret.-Recent
Trochacea							
Trochidae	s <i>Calliotoma incerta</i>	Mar. b.	Recent	Pupillacea			
Turbinidae	h? <i>Scaevola</i>	Mar. b.	Jur.	Vertiginidae	s Some <i>Vertigo</i>	Land	Recent
	p <i>Helicocryptus</i> , <i>Pseudoliotina</i>	Mar. b.	Jur.-Cret	Chondrinidae	s Some <i>Gastrocopta</i>	Land	Recent
Order Mesogastropoda				Pupillidae	s Some <i>Pupilla</i>	Land	Recent
Valvatidae	p Some Valvatidae	Fr.	Ord.-Recent	Enidae	s Some <i>Jaminia</i>	Land	Recent
Viviparidae				Clausiliidae	s Clausiliidae	Land	Recent
Plidae	h <i>Marissa cornuarietis</i>	Fr.	Recent	Corillidae	h? <i>Plectophyllis</i>	Land	Recent
Atlantidae	p ¹¹ <i>Lanistes</i>	Fr.	Tert.-Recent		p Some <i>Corilla</i>	Land	Recent
Loxonematacea	p Most Atlantidae	Mar. pl.	Cret.-Recent	Ariophantacea			
Zygopleuridae	s <i>Virgella</i> , <i>Allostrophia</i>	Mar. b.	Tri.-Jur.	Ariophantidae	p <i>Coxia</i>	Land	Recent
Rissoacea					s? <i>Dyakia</i> , <i>Bertia</i> , <i>Ariophanta</i>	Land	Recent
Hydrobiidae	p Cochliopinae	Fr.	Recent	Rhytididae	p <i>Diplomphalus</i>	Land	Recent
Vitrinellidae	p Many genera	Mar. b.	Cret.-Recent	Endodontidae	p <i>Hirasea</i>	Land	Recent
Omalogyridae	p Whole family	Mar. b.	Recent	Zonitidae	p Some <i>Helicodiscus</i>	Land	Recent
Triphoridae	s Triphoridae	Mar. b.	Cret.-Recent	Systrophidae ²²	p <i>Polygyra</i>	Land	Recent
Cyclophoridae	s Many <i>Diplommatina</i>	Land	Recent	Polygyracea			
Order Neritogastropoda				Polygyridae	p Some <i>Polygyra</i>	Land	Recent
Helicinidae	p <i>Ceratodiscus</i>	Land	Dev.-Recent	Ammonitellidae	p <i>Ammonitella</i> , <i>Spelaodiscoides</i> ²²	Land	Recent
Neogastropoda				Helicacea			
Muricea				Camaenidae	s Some <i>Camaena</i> , <i>Syndromus</i> , <i>Eulota</i> , and <i>Amphidromus</i>	Land	Recent
Buccinidae	s Some <i>Volutopsius</i> and <i>Neptunea</i>	Mar. b.	Tert.-Recent				
Melongenidae	s Some <i>Busycon</i>	Mar. b.	Tert.-Recent				

Mar., marine; b., benthic; pl., planktonic; Fr., freshwater; Cam., Cambrian; Tri., Triassic; Dev., Devonian; E. Ord., Early Ordovician; Perm., Permian; Cret., Cretaceous; Jur., Jurassic; Mio., Miocene; Plio., Pliocene; Quat., Quaternary.

* Most Macluritacea and many Euomphalacea are hyperstrophic, but their orientation and mode of life are poorly understood.

populations in which stabilising selection has been relaxed⁶. Left-handedness in normally dextral snails is often associated with aberrant shell shape^{12,23-25}. The general reduction in dispersability associated with loss of a planktonic stage may lead to a higher probability of geographic isolation of small populations. The greater the number of such populations, the higher is the overall probability for reversed symmetry to arise. Reversed symmetry is thus most likely and most widespread in groups such as the Neogastropoda, containing many lineages with direct development, and especially the hermaphroditic and often locally distributed Stylommatophora.

Several anomalies in the distribution of gastropod coiling types are unexplained. All sinistral neogastropods occur or are inferred to have lived in marine soft-bottom environments, none being known from hard substrata. Among land Stylommatophora, sinistral coiling is rare in Africa, Australia and the Americas, but quite common in Europe and especially South Asia and the Pacific islands. The near absence of sinistral species in the three or four stocks of terrestrial prosobranchs, and the evolution of only one small lineage of planispiral forms in this group, are additional enigmas.

Intense stabilising selection may preclude the evolution of planispiral, hyperstrophic, and sinistral gastropods from dextral ancestors because the mechanisms altering coiling direction are normally selected against. This does not mean that all coiling types are adaptively interchangeable⁶. It has been suggested¹² that hyperstrophy in benthic snails renders crawling more difficult than in dextral or sinistral types. The benefit of improved locomotory efficiency with planispiral compared with asymmetrical coiling may in some cases out-

weigh the disadvantage of a weaker shell. Relaxation of selection for strong shells or for efficient locomotion could thus allow other, sometimes opposing, selective pressures to fix and maintain new coiling types.

GEERAT J. VERMEIJ

Department of Zoology,
University of Maryland,
College Park, Maryland 20742

Received January 30; revised February 11, 1975.

- Vermeij, G. J., *Bull. Mar. Sci.*, **23**, 351-386 (1973).
- Flower, R. H., *Evolution*, **9**, 244-260 (1955).
- Arkell, W. J., Kummel, L., and Bright, C. W., *Treatise on Invertebrate Paleontology*, Part 1 (Ammonoidea) (University of Kansas Press, Lawrence, 1957).
- Nicol, D., *Jl Washington Acad. Sci.*, **48**, 56-62 (1958).
- Knight, J. B., *Smithsonian Misc. Coll.*, **117**, (13), 1-56 (1952).
- Vermeij, G. J., *Syst. Zool.*, **22**, 466-477 (1973).
- Robertson, R., *Nautilus*, **87**, 88 (1973).
- Crofts, D. R., *Proc. Zool. Soc. Lond.*, **125**, 711-750 (1955).
- Underwood, A. J., *Mar. Biol.*, **17**, 341-349 (1972).
- Raven, C. P., *Morphogenesis: the analysis of molluscan development*, second ed., (Pergamon, Oxford, 1966).
- Lang, A., *Lehrbuch der vergleichenden Anatomie der wirbellosen Tiere* (edit. by Hescheler, K.), (Fischer, Jena, 1900).
- Robertson, R., and Merrill, A. S., *Veliger*, **6**, 76-79 (1963).
- Harry, H. W., *Malacologia*, **1**, 355-385 (1964).
- Hubendick, B., *Trans. Zool. Soc. Lond.*, **28**, 453-542 (1955).
- Raup, D. M., *J. Paleont.*, **40**, 1178-1190 (1966).
- Leigh, E. G., *Adaptation and Diversity* (Freeman, Cooper, San Francisco, 1971).
- Vermeij, G. J., *Evolution*, **28**, 656-664 (1974).
- Schaeffer, B., and Rosen, D. E., *Am. Zool.*, **1**, 187-204 (1961).
- Stevic, N., *Syst. Zool.*, **20**, 331-340 (1971).
- Knight, J. B., Cox, L. R., Keen, A. M., Batten, R. L., Yochelson, E. L., and Robertson, R., *Treatise on Invertebrate Paleontology*, Part 1 (Mollusca) (University of Kansas Press, Lawrence, 1960).
- Abbott, R. T., *American Seashells*, second ed. (Van Nostrand Reinhold, New York, 1974).
- Taylor, D. W., and Sohl, N. F., *Malacologia*, **1**, 7-32 (1962).
- Reigel, N. J., *Nautilus*, **76**, 36-37 (1962).
- Bickel, D., *Nautilus*, **79**, 107-108 (1966).
- Janssen, A. W., *Basteria*, **30**, 8-10 (1966).

Prolonged eggshell thinning caused by DDE in the duck

ALTHOUGH eggshell thinning induced by 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene (DDE)—has been studied in many species (see review by Cooke¹), the long term effects of this pesticide have not been investigated. Its duration of action is of great environmental significance since it will determine the effect of any pesticide ingested before the breeding season. In this respect, the finding of continuing high levels of DDE and thin eggshells in the Alaskan peregrine falcon (*Falco peregrinus*) in areas free from direct spraying² argues for a prolonged effect, but direct controlled studies have been lacking. In fact, 18 d is the longest period to date during which thinning has been monitored (in mallards given single oral doses of 500–2,000 mg DDE kg⁻¹) and little recovery of shell thickness was observed³.

We report here the changes in eggshell thickness in the white Peking duck (a domesticated mallard) during 27 weeks following a brief exposure to dietary DDE. Ducks approximately 6 months old were divided into experimental and control flocks (three birds in each) and maintained as before⁴. The experimental flock was fed 250 p.p.m. DDE for 10 d and then control duck feed (provided by Agway Inc.) for the rest of the experiment. A rough calculation from the rate of food consumption shows that experimental ducks (body weight 5–6 kg) ingested about 0.5 g DDE during the 10 d. Approximately 2 months later, when all birds began laying, eggs were collected daily and frozen, and the shells were cut at the equator, that is, approximately midway between the ends. Eggshells were washed in warm water and dried overnight at 45 °C. Twenty measurements of shell thickness, ten per half, were made near the equator of the egg using an Ames micrometer. Each bird produced an average of three or four eggs per week, except for a moulting period of about 7 weeks, when none was laid (weeks 14–20, Fig. 1). As Fig. 1 shows, eggshells from ducks fed DDE were approximately 20% thinner than controls, which agrees with the maximum thinning

reported before⁴. Recovery of shell thickness was slow, being less than half-way at the end of 27 weeks. DDE residues in the yolks were analysed as previously described⁵. Initially, levels were greater than 150 p.p.m. dry weight (Fig. 1) and after 27 weeks, residue values had declined only to about 90 p.p.m. In contrast, levels of DDE residues in a control egg yolk (from week 5) were 0.23 p.p.m. Based on the values of residues shown in Fig. 1, we have calculated the approximate amount of DDE lost by a bird through its eggs. Using a value of 150 µg DDE per g of dried yolk, and assuming that 75 eggs were laid during the 27 weeks, each containing 18 g of yolk (dried weight), roughly 0.2 g DDE or 40% of the original dose would be lost. This calculated clearance parallels the measured decrease in residue levels, that is, approximately 40% decrease in yolk DDE in 25 weeks (Fig. 1). Likewise, Tucker and Haegele⁶ found significant loss of DDT through the eggs of mallards. Thus, in the duck, the egg is the major route of excretion for DDE. Naturally, in the wild, the loss would be much less since many fewer eggs are laid.

Earlier work on the white Peking duck demonstrated the rapid onset of eggshell thinning, which is essentially complete within 4 d of exposure to 40 p.p.m. of DDE in the diet⁴. Our study now shows that although the egg is a major route of excretion of DDE, loss through this mechanism is relatively slow, as is recovery of eggshell thickness. Thus, DDE-induced eggshell thinning has a rapid onset and is essentially irreversible.

This study was supported by the US Public Health Service and the US National Science Foundation.

DAVID B. PEAKALL

Cornell University,
Ithaca, New York 14850

DAVID S. MILLER
WILLIAM B. KINTER

Mount Desert Island Biological Laboratory,
Salsbury Cove, Maine 04672

Received November 11, 1974; revised February 10, 1975.

¹ Cooke, A. S., *Environ. Pollution*, **4**, 85–152 (1973).

² Peakall, D. B., Cade, T. J., White, C. M., and Haugh, J., *Pesticide Monitoring*, **J.** (in the press).

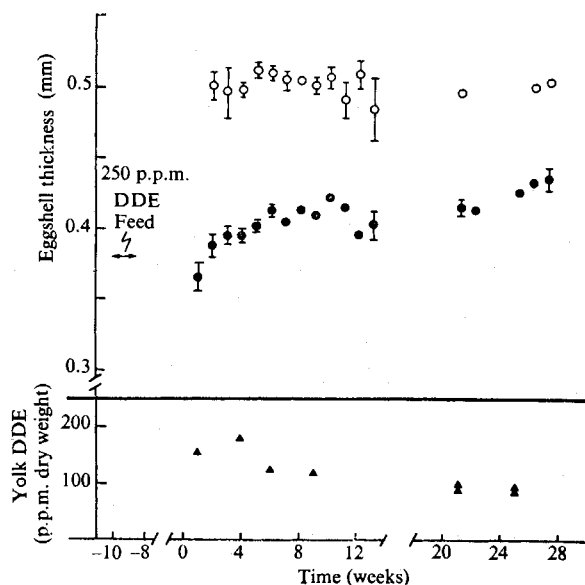
³ Haegele, M. A., and Tucker, R. K., *Bull. environ. Contamin. Toxicol.*, **11**, 98–102 (1974).

⁴ Peakall, D. B., Lincer, J. L., Risebrough, R. W., Pritchard, J. B., and Kinter, W. B., *Comp. gen. Pharmacol.*, **4**, 305–313 (1973).

⁵ Cade, T. J., Lincer, J. L., White, C. M., Roseneau, D. G., and Swartz, L. G., *Science*, **172**, 955–957 (1971).

⁶ Tucker, R. K., and Haegele, M. A., *Bull. environ. Contamin. Toxicol.*, **5**, 191–149 (1970).

Fig. 1 Eggshell thickness as function of time for control (○) and DDE-fed (●) white Peking ducks. Experimental birds were fed 250 p.p.m. DDE only during the time indicated. Each point represents the mean thickness for all eggs collected during the 1 week (approximately 11 eggs per flock per week). Variability, when large enough, is shown as standard error bars. Also shown are DDE residues in randomly selected egg yolks from ducks fed DDE. Values are given as p.p.m. dry weight in individual egg yolks. (These values can be converted to p.p.m. wet weight by multiplying by 0.23 or to p.p.m. lipid weight by multiplying by 1.7.)



Acclimation in arctic lichens

USING a new gas exchange method, we have followed the seasonal response of net assimilation rate (NAR) in Arctic lichens and have found rapid acclimation to temperature, light and thallus moisture content. This suggests that these organisms can adjust reversibly their patterns of physiological response to suit the atmosphere of their rather stressful habitats.

Although there is increasing evidence of temperature acclimation of net assimilation rate (NAR) in various plants, both in the field^{1–3} and as responses induced under laboratory conditions^{4,5}, investigations with higher plant systems are complicated by a combination of acclimatory response with variations of NAR resulting from seasonal production of new shoots, flowering and senescence of leaves. Lichens do not undergo seasonal growth of this sort and thus are an excellent system in which to study acclimation.

Infrared gas analysis can be used to measure NAR of plant tissue as a change in CO₂ concentration within a flowing gas stream before and after it passes over the experimental plant material, which is enclosed in a special assimilation chamber. We have found that the gas flow networks and environmental control systems which are necessary are too costly and complex to permit use of more than one or two assimilation chambers. Since only one replicate sample can be included in the chamber at once, any adequately replicated experiment is very time

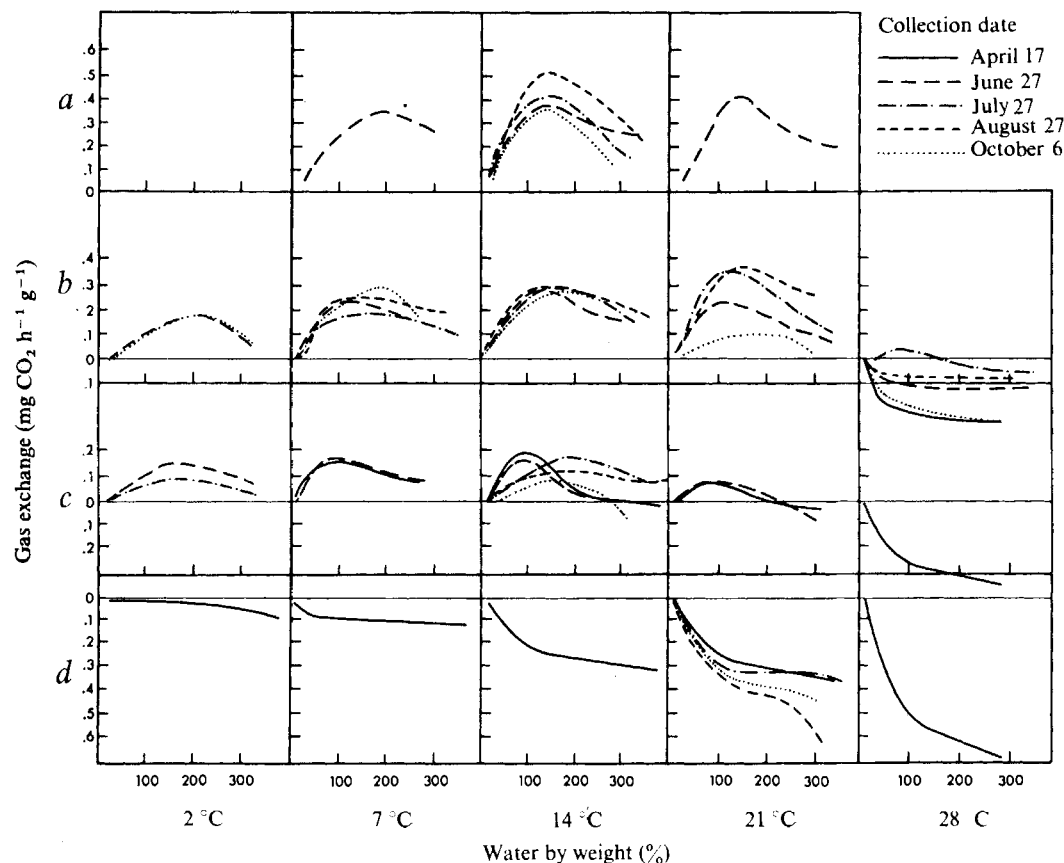


Fig. 1 Seasonal response of NAR in *Cetraria nivalis*. Rates of assimilation ($\mu\text{Einstein m}^{-2} \text{s}^{-1}$): a, 250; b, 150; c, 50; d 0. Each line is the mean of four or five replicates with s.e.m. less than $\pm 0.02 \text{ mg CO}_2 \text{ h}^{-1} \text{g}^{-1}$. All collection dates are from 1974.

consuming. Although such methods have been used to investigate gas exchange characteristics of lichens³⁻⁵, the time required to study the basic responses to light, temperature and moisture has frustrated many attempts to demonstrate rapid acclimation in these plants^{6,7}.

We have conducted parallel experiments on various lichens from Arctic tundra using both a standard ventilated system⁹ and a new method involving sealed gas exchange cells. We found that unlike the situation with higher plants, ventilation does not influence rates of CO_2 exchange by lichens, and that the concentration of CO_2 around the thallus may fall as low as 150 p.p.m. without any change in the rate of depletion of CO_2 from the vessel. Our results suggest that in these lichens, rates of net assimilation are so low that no strong CO_2 gradients appear within the thallus. It is also important that gas exchange can occur freely across the thallus since stomata are absent. Small samples of lichen (0.25 g) were placed in clear gas exchange cells, sealed and incubated under specified conditions of temperature and radiation within a walk-in growth chamber. After incubation for a specified time the change in CO_2 concentration within the gas exchange cells was measured by injection of a sample into a carrier gas stream flowing through an infrared gas analyser. The value obtained was standardised to yield a value of $\text{mg CO}_2 \text{ h}^{-1} \text{g}^{-1}$ dry weight of the thallus. This was plotted against the water content of the thallus—the primary controlling factor of carbon assimilation in lichens—which was measured by weighing before and after each incubation. Rates of net assimilation for *Cetraria nivalis* and *Parmelia caperata* found using this system were the same as those previously obtained by standard methods^{4,9,10}.

We have used our system to follow the seasonal response of NAR to three variables in *Cetraria nivalis* collected from Arctic tundra at East Pen Island, Hudson Bay. In Fig. 1 data appear as a 'physiological matrix' of responses to all thallus moisture levels, five temperatures and four light levels, measured five times during 1974. Basic gas exchange response changed con-

tinuously throughout 1974. The extent of these changes was considerable: for example levels of assimilation achieved at $250 \mu\text{Einstein m}^{-2} \text{s}^{-1}$ (1 einstein is 1 Avogadro's number (6.023×10^{23}) of photons, and 1 $\mu\text{Einstein}$ is approximately equal to 50 lx) and 14°C in June were only about 15% higher than at $150 \mu\text{Einstein m}^{-2} \text{s}^{-1}$. Conversely, by July and August, levels of assimilation at $250 \mu\text{Einstein m}^{-2} \text{s}^{-1}$ were 60% higher than at $150 \mu\text{Einstein m}^{-2} \text{s}^{-1}$. By October, after the first snowfall, the maximum NAR at $250 \mu\text{Einstein m}^{-2} \text{s}^{-1}$ was again only 15% higher than at $150 \mu\text{Einstein m}^{-2} \text{s}^{-1}$.

At $50 \mu\text{Einstein m}^{-2} \text{s}^{-1}$ and 14°C in April NAR in *Cetraria nivalis* was optimal at 100% water by weight; by July and August the response to thallus water content was much flatter, with an increase in NAR at higher levels of thallus water content. By October, the response had decreased and indicated a return towards the response found in the spring.

At $150 \mu\text{Einstein m}^{-2} \text{s}^{-1}$, the optimal temperature for NAR was near 14°C in June, near 21°C in July and had shifted back towards 7°C in October. In October NAR at 21°C was about 70% of its value in late August.

It is not known whether the capacity for rapid acclimation demonstrated here is restricted to lichens collected from extreme environments such as East Pen Island or whether temperate or tropical species have equal facilities for acclimation. This could be tested by comparing ecotypes of species such as *Cladonia mitis*, the distribution of which extends from high latitude sites like East Pen Island to temperate pine woodland. Although samples of this species collected in Wisconsin¹¹ showed no significant ecotypic or seasonal variation in response to light intensity, very different patterns can be expected from this species collected from tundra sites.

Our experimental system, with lichens as the experimental material, provides evidence that acclimation occurs very rapidly, is not restricted to shifts in temperature optima and may involve changes in response to light intensity and water content. We are investigating how the assimilation rate of lichens

acclimated to cold, dark and dry conditions is reversed after transfer to warm, bright and wet environments. On the basis of available evidence we suspect that such acclimation can be induced in a matter of days.

D. W. LARSON
K. A. KERSHAW

Department of Biology,
McMaster University,
Hamilton, Ontario L8S 4K1

Received November 19, 1974; revised February 6, 1975.

- ¹ DePuit, E. J., and Caldwell, M. M., *Am. J. Bot.*, **60**, 426–435 (1973).
- ² Billings, W. D., Godfrey, P. J., Chabot, B. F., and Bourque, D. P., *Arctic Alpine Res.*, **3**, 277–289 (1971).
- ³ Lechowicz, M. J., and Adams, M. S., *Can. J. Bot.*, **52**, 411–422 (1974).
- ⁴ Kallio, P., and Heinonen, S., *Rep. Kevo Subarctic Res. Stat.*, **8**, 63–72 (1971).
- ⁵ Smith, E. M., and Hadley, E. B., *Arctic Alpine Res.*, **6**, 13–28 (1974).
- ⁶ Lechowicz, M. J., and Jordan, W. P., and Adams, M. S., *Can. J. Bot.*, **52**, 505–573 (1974).
- ⁷ Lange, O. T., *Flora Jena*, **158**, 324–359 (1969).
- ⁸ Sestak, Z., Jarvis, P. G., and Catsky, J., in *Plant Photosynthetic Production, Manual of Methods*, (edit. by Sestak, Z., Catsky, J., and Jarvis, P. G.), 1–38 (Junk, The Hague, 1971).
- ⁹ Harris, G. P., *J. Ecol.*, **59**, 441–452 (1971).
- ¹⁰ Bliss, L. C., and Hadley, E. B., *Am. J. Bot.*, **51**, 870–874 (1964).
- ¹¹ Lechowicz, M. J., and Adams, M. S., *Ecology*, **54**, 413–419 (1973).

Petroleum-degrading achlorophyllous alga *Prototheca zopfii*

ONE of the objectives of this research programme is to enumerate and identify petroleum-degrading microorganisms occurring in Chesapeake Bay, to assess the potential of these microorganisms to degrade petroleum *in vitro* and *in situ* and to determine the types of petroleum degraded¹. We are sampling two locations in Chesapeake Bay at monthly intervals. One is Colgate Creek, an oil-contaminated site in Baltimore Harbour, the other is an area south of Parson's Island in Eastern Bay, a non-polluted shellfish harvesting region of Chesapeake Bay. The presence of petroleum in Colgate Creek and its absence in Eastern Bay has been confirmed by computerised low-resolution mass spectrometry analysis of benzene extracts of sediments collected at both sites^{2,3}. We have routinely isolated petroleum-degrading bacteria, yeasts and filamentous fungi from Colgate Creek and Eastern Bay, but during April and May 1973, and again in 1974, an unusual organism, believed to be an algal species, was isolated from sediment of Colgate Creek. Corresponding isolates have not been isolated from Eastern Bay.

Routine environmental parameters measured during April and May 1973 and 1974 yielded similar data for both years:

air temperature 18–21 °C; dissolved oxygen, 9–12 p.p.m.; water temperature, 12–17 °C; pH 7–8, and salinity, 6–8 ‰. The unusual organism was isolated at a time of year when significant petroleum biodegradation occurred in Colgate Creek⁴.

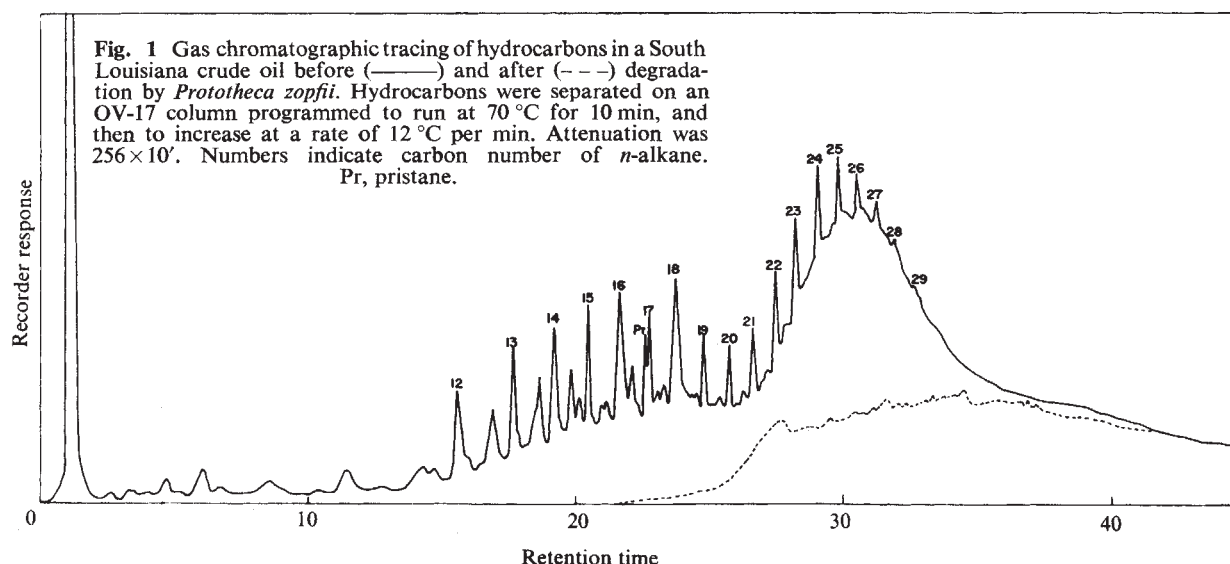
The organism was isolated, in axenic culture, on silica gel oil medium, supplemented with streptomycin and tetracycline which inhibited growth of petroleum-degrading bacteria⁵. The significant feature of our isolate is that it degrades petroleum hydrocarbons. When the organism was grown in 100 ml estuarine salts solution¹ overlaid with 1% (v/v) of a South Louisiana crude oil, we observed significant degradation of the oil, after 30 d of incubation at 25 °C in quiescent culture, compared with the uninoculated, weathered control sample of oil (Fig. 1).

The isolate has been identified as *Prototheca zopfii* (Table 1), following the description provided by Arnold and Ahearn⁶, as well as by photomicroscopy (Fig. 2), and electron microscopy (Fig. 3), as recommended by Nadakavukaren and McCracken⁷ for differentiating *Prototheca* from fungi. As well as crude oil, the isolate utilised petroleum hydrocarbons in a 17-component mixed hydrocarbon substrate⁸, after 14 d of incubation under the conditions described in Table 2. The mixed hydrocarbon substrate permitted determination of the

Table 1 Physiological characteristics of *Prototheca zopfii*

Assimilation of carbon compounds:			
Glucose	+	L-arabinose	—
Galactose	+	D-arabinose	—
L-sorbose	—	D-ribose	—
Maltose	—	L-rhamnose	—
Sucrose	—	Glycerol	+
Cellobiose	—	iso-erythritol	—
Trehalose	—	Adonitol	—
Lactose	—	Dulcitol	—
Melibiose	—	D-mannitol	—
Raffinose	—	D-sorbitol	—
Melezitose	—	α -methyl-D-glucoside	—
D-xylose	—	Salicin	—
		Inositol	—
Assimilation of potassium nitrate: negative			
Growth at 37 °C: positive			
Growth at 10 °C: negative			

Results of assimilation tests after 28 d incubation. Yeast nitrogen base (Difco) plus 0.5% carbon source. Yeast carbon base (Difco) plus 0.5% potassium nitrate. Growth at various temperatures: YM agar (Difco), 7 d.



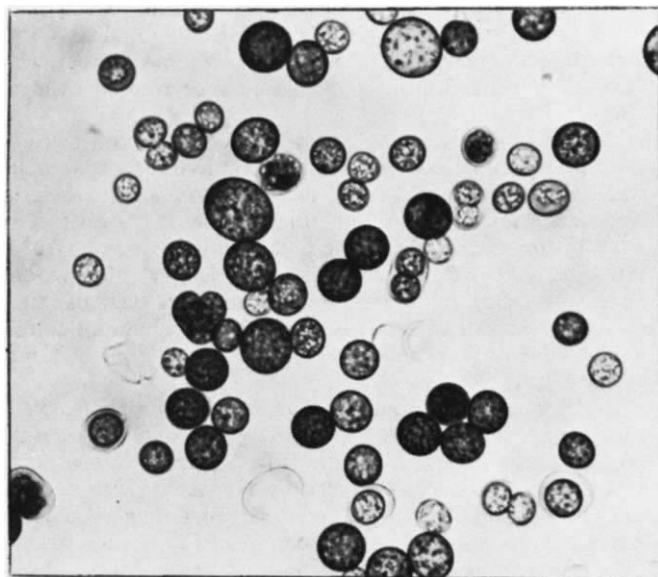


Fig. 2 Photomicrograph of the petroleum-degrading *Prototheca zopfii* ($\times 750$).

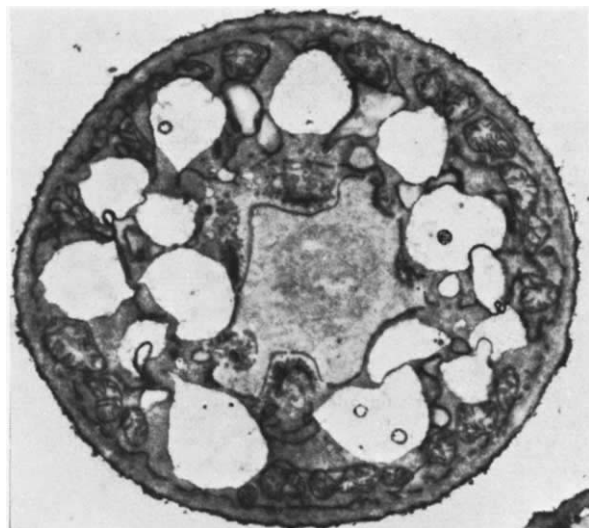


Fig. 3 Electron micrograph of a thin section through a *Prototheca zopfii* cell showing internal structures typical of this species⁷. A 48-h culture grown in 1% glucose-estuarine salts medium was fixed in 1% sodium permanganate for 8 h, dehydrated through a graded ethanol series, embedded in Epon, sectioned and poststained with uranyl acetate and lead citrate. The thin sections were examined and photographed with an RCA EMU-3F electron microscope ($\times 9,500$).

Table 2 Degradation of hydrocarbons in mixed hydrocarbon substrate by *Prototheca zopfii*

Hydrocarbon	% Remaining		Individual hydrocarbon degraded (%)
	Weathered sample	Degraded sample	
Cumene	1.58	0.02	98.74
<i>n</i> -Decane	7.66	0.01	99.88
<i>n</i> -Undecane	7.59	0.46	93.94
Naphthalene	0.65	0.01	99.99+
<i>n</i> -Dodecane	7.76	2.33	69.98
<i>n</i> -Tridecane	8.20	4.06	50.49
<i>n</i> -Tetradecane	8.68	5.10	41.25
<i>n</i> -Pentadecane	11.28	7.37	34.67
<i>n</i> -Hexadecane	9.03	5.66	37.33
<i>n</i> -Heptadecane	13.58	8.62	36.53
Pristane	0.86	0.47	45.35
<i>n</i> -Octadecane	8.76	5.38	38.59
<i>n</i> -Nonadecane	8.45	5.02	40.60
<i>n</i> -Eicosane	10.82	6.50	39.93
Phenanthrene	0.41	0.24	41.47
1,2-Benzanthracene	0.47	0.28	40.43
Perylene	1.85	1.17	36.76

type of petroleum hydrocarbons utilised by this unusual algal isolate.

Growths of a *Prototheca* sp. on *n*-alkanes has been described⁹, but this is the first report of the degradation of oil by an algal species. Although the significance of algae in this process has yet to be assessed, they were previously not considered capable of degrading petroleum hydrocarbons. The role of algae in the microbial degradation of oil must now be examined.

This research was supported by an Office of Naval Research contract and a grant from the US National Science Foundation.

J. D. WALKER
R. R. COLWELL
Z. VAITUZIS

Department of Microbiology,
University of Maryland,
College Park, Maryland 20742

S. A. MEYER

Department of Protistology,
American Type Culture Collection,
Rockville, Maryland 20852

Received October 17, 1974; revised February 11, 1975.

- 1 Walker, J. D., and Colwell, R. R., in *API/EPA/USCG Conf. Prevention and Control of Oil Spills*, 685-691 (American Petroleum Institute, Washington, DC, 1973).
- 2 Walker, J. D., and Colwell, R. R., *Appl. Microbiol.*, **27**, 285-287 (1974).
- 3 Walker, J. D., Colwell, R. R., Hamming, M. C., and Ford, H. T., *Bull. Env. Contam. Toxicol.*, **E3**, 245-248 (1975).
- 4 Walker, J. D., and Colwell, R. R., *Microbial. ecol.*, **1**, 63-95 (1974).
- 5 Walker, J. D., and Colwell, R. R., *Prog. Water Technol.* (in the press).
- 6 Arnold, P., and Ahearn, D. G., *Mycologia*, **64**, 265-275 (1972).
- 7 Walker, J. D., and Colwell, R. R., *J. Phycol.*, **9**, 113-116 (1973).
- 8 Walker, J. D., and Colwell, R. R., *Appl. Microbiol.*, **28**, 1053-1060 (1974).
- 9 Kockova-Kratochvilova, A., and Havelkova, M., *Z. Allg. Mikrobiol.*, **14**, 123-124 (1974).

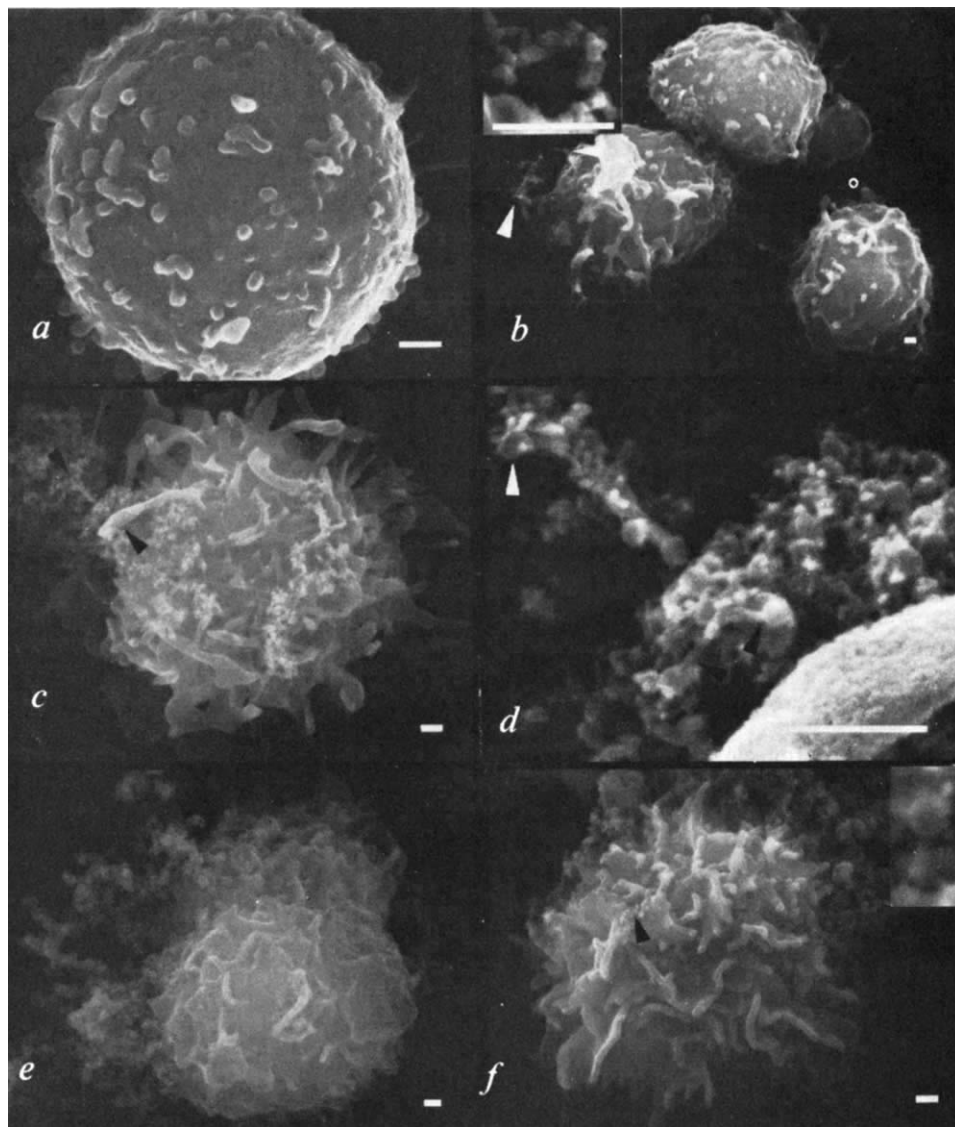
Multiple labelling technique used for kinetic studies of activated human B lymphocytes

IDENTIFICATION of distinct cell surface receptors which could be viewed in the scanning electron microscope would allow unambiguous identification of subpopulations of cells and quantitation of receptors on individual cells as well as permitting kinetic studies of the membrane changes following the binding of a ligand with its receptor. Labelling techniques using one or two markers have been developed for transmission electron microscopy (TEM)^{1,2}. But scanning electron microscopy (SEM) offers several advantages over TEM for analysing membrane receptors in that it does not require serial sectioning of material³ and therefore allows rapid viewing of the surfaces of large numbers of intact cells with a minimum of preparation. Here I describe new labelling techniques for SEM using three distinct markers conjugated to antibodies specific for the immunoglobulins IgA, IgG and IgM.

Previous labelling techniques developed for SEM⁴⁻⁶ have several limitations. First the techniques use only one marker, restricting visualisation to only one type of receptor⁴⁻⁶. Second, with one exception⁴, the techniques utilise a large, spherical (130-230 nm diameter) marker which obscures the cell surface preventing precise localisation and quantitative analyses of receptors. Third, the marker has a hydrophobic surface which makes it stick nonspecifically to many surfaces and molecules, and the absorbed protein can dissociate from the marker at physiological salt concentration and pH (ref. 6).

This study was undertaken to determine the feasibility of employing a tri-label technique for SEM. The SEM markers, which were selected for their size and distinctive shapes, were the cylindrical molecule, keyhole limpet haemocyanin (KLH), the icosahedral non-plaque forming simian virus 40 (SV40) and the hexagonal-headed T2 bacteriophage (T2). Antibodies specific for immunoglobulins (Ig) A, G or M were conjugated to individual markers¹¹, and human B cells carrying Ig were then selectively labelled with these reagents. Surface changes associated with activation were followed during kinetic studies to try to resolve the controversy concerning the appearance

Fig. 1 Lymphocytes were separated from heparinised human blood on "Lymphoprep" (Neygaard and Co.)¹², washed three times in a 1:1 mixture of the decomplexed, autologous serum and PBS, pH 7.4 and resuspended in Medium 199, pH 7.4, at a concentration of 1×10^6 cells ml^{-1} . Macrophages were removed by adherence to glass. Sheep red blood cell (SRBC) rosettes were formed by a modification of previous techniques^{10,14}. Briefly, autologous heat inactivated serum, which had been absorbed against SRBC at 4 and 37 °C, was added to the lymphocyte suspension so that the final serum concentration was 20%, and the cells were incubated for 60 min at 37 °C. Washed SRBC were then added and the suspension layered on "Lymphoprep" at 4 °C and centrifuged at 400g for 15 min at 24 °C. The non-rosetting B lymphocytes which comprised about 20% of the total peripheral blood lymphocytes were collected from the top of the gradient, washed three times with Medium 199, and 0.1 ml pipetted on to 12 replicate glass coverslips inside plastic Petri dishes (Falcon). All three SEM marker conjugates were added to each coverslip. Controls consisted of unconjugated markers added to six coverslips. For kinetic studies, cells were incubated for 2 min at 37 °C or for 15, 30 or 45 min at 4 °C, fixed for 30 min with 1% GA and then for 4 h with 2.8% GA¹⁰, dehydrated¹⁰, CO₂ critical point dried, and coated with 5 nm of gold-palladium. Specimens were viewed for between 4 and 8 h in an Hitachi HHF 2R SEM operated at 20 kV. A minimum of 2,000 cells in each preparation were studied. *a*, Control untreated B cell. The surface is not labelled and is smooth except for microvilli. *b*, Elongated macrovilli are labelled with KLH-anti-IgA (between arrows; 2 min after label). Inset shows KLH as a rectangle when viewed sideways and as a circle when viewed on end (high magnification of area adjacent to right arrow $\times 31,350$.) *c*, At low magnifications, T2-anti-IgM appears as 'fuzz' on the cell surface which is covered with elongated macrovilli ($\times 5,225$). A 'cap' has formed on the upper left of the cell (15 min after label). *d*, High magnification of the area between the arrows in *c* ($\times 37,400$) demonstrating T2 (black arrows indicate junction between the head and tail of T2). White arrow indicates another T2 of which both head and tail are visible. Because of the density of marker, generally only T2 heads or tails are visible as in the upper right of the micrograph. Apparently, the phage was osmotically shocked during the conjugation procedures because most of the heads are round rather than hexagonal. *e*, SV40-anti-IgG labelled B cell with undulate surface (15 min after label). *f*, B cell labelled with T2-anti-IgM at 30 min. Lamellae cover the surface, and the marker which is too dense to count has 'capped' on the upper part of the cell. Marker is also in scattered areas on the surface. Inset is high magnification ($\times 75,900$) of T2 from areas at the tip of the arrow. Magnification line equals 500 nm.



of human B cells under SEM. Previously, human B cells have been reported to be either highly undulate⁸, highly villous^{8,9} or sparsely villous¹⁰, and this controversy has precluded the use of SEM as a simple method for distinguishing between B and T cells.

Markers were fixed in 1% glutaraldehyde (GA) for 2 h to preserve their structure and then dialysed overnight against phosphate buffered saline (PBS), pH 6.9. One ml of IgG fraction of goat antiserum directed against human IgM (3.4 mg ml^{-1}), IgG (15.2 mg ml^{-1}) or IgA (7 mg ml^{-1}) was added to 2 ml of T2 (Miles Lab), SV40 or KLH, respectively, so that the ratio of antibody molecules to marker was 1:1. The antisera were specific for the Ig against which they were made as determined by immunoelectrophoresis and double diffusion in agar. An 8% solution of GA was added dropwise with agitation until its final concentration was 0.1%. Marker labelled antisera preparations were then incubated at 20 °C for 2 h with agitation¹¹, and dialysed against PBS, pH 7.4, overnight at 4 °C. Conjugates were purified by column chromatography on Agarose A 1.5 m (Biogel 100-200 mesh). The first millilitre containing aggregates

was discarded. The conjugate was present in the first peak, unconjugated marker in the second, and unconjugated gamma globulin in the third.

In preliminary experiments, it was found that the amount of a marker present on a labelled cell was the same at a given incubation time irrespective of whether 1, 2 or 3 markers were used whereas the percentage of B cells labelled increased as more than one marker was used ($1 < 2 < 3$). The relative number of cells labelled with T2-anti-IgM, SV40-anti-IgG, or KLH-anti-IgA varied with the individual whose cells were tested (some had a preponderance of B cells carrying IgM while others had a preponderance of IgG cells). No cells were double labelled. When B cells were treated with a mixture of unlabelled and marker labelled anti-Ig, labelling was drastically reduced. These results confirmed the specificity of the marker-anti-Ig conjugates. "Stacking" of the marker was observed when conjugates were added to live cells but not when the marker was added to cells prefixed with 1% GA and washed in buffer. Moreover, aggregates were not observed when conjugates alone were scanned. This shows that the "stacking"

is not a result of the presence of aggregates in the conjugates. Rather it requires a viable cell membrane, suggesting that membrane fluidity may be responsible.

Incubation times and temperatures for the kinetic studies were selected on the basis of results from preliminary experiments using 1, 3, 6, 9, 12, 15 or 30 min at 37 °C, and 5, 15, 30, 45 or 60 min at 4 °C. All morphological changes observed with SEM occurred within 6 min at 37 °C which is sooner than can be detected with light microscopy. Since this was much too rapid to enable careful analysis of these cellular changes, most experiments were performed at 4 °C to slow membrane movement. It was found initially that cells incubated at 4 °C for 60 min, but not 45 min, showed subtle changes in surface ultrastructure including occasional contraction and collapse of some villi and lamellae; therefore 45 min was selected as the maximum incubation time at 4 °C.

B cells in the untreated control preparations had fewer, than 100 microvilli per cell, evenly distributed on the surface (Fig. 1a). Within 2 min of addition of marker-antiserum conjugates at 37 °C, the microvilli on labelled cells had migrated to one pole of the cell forming a 'cap', while the elongated macrovilli and lamellae formed at the other (Fig. 1b). An average of 10 markers were visible on each labelled cell. The inset on Fig. 1b shows clearly the marker, KLH, on the elongated macrovilli. KLH is also present on the 'capped' microvilli. By 15 min, elongated macrovilli and lamellae covered the surfaces of 70% of the B cells (Fig. 1c and d) and an average of 300 markers were visible on each labelled cell. Figure 1e shows an IgG-producing B cell labelled with SV40 anti-human IgG. Within 30 min, the surfaces of 50% of the labelled B cells were extensively covered with lamellae, and the marker had again 'capped' (Fig. 1f). No difference was observed in the surface appearance of IgA, IgG and IgM cells. When kinetic experiments were repeated using unconjugated Ig, the same sequence of membrane changes were observed indicating that these are not artefacts resulting from binding of a heavy marker.

These results indicate that unstimulated B cells have microvilli on their surface but do not have elongated macrovilli or lamellae. When activated by anti-Ig receptor reagents, B cells respond first with the formation of elongated macrovilli and then lamellae. Simultaneously with the appearance of macrovilli, the number of Ig receptors present on the surface increases by at least tenfold (from approximately 10 to 300 receptors per cell). This is a minimum estimate because (a) only 1/2 to 3/4 of any individual cell can be seen at any one time with SEM, (b) the marker may be bound in layers or may have entered the cell and (c) some of the marker may have been removed during critical point drying. In spite of these limitations, SEM analysis of surface events, and binding of labelled molecules in particular, is more accurate than TEM analysis which depends on the plane of section (that is, a typical 30 to 60 nm section of a cell may or may not be through an area where the receptors are present in a proportion representative of the whole cell).

Some authors have shown previously that human B cells have relatively few microvilli³ while others have contended that they have highly villous¹² or undulating surfaces¹. As is evident from the results of these experiments, the surface appearance of B cells depends on their stage of activation. Those who described B cells as having relatively few villi had incubated them in PBS without antibody or complement^{10,13}, whereas those who described them as "highly villous", used non-autologous pooled Type AB serum which had not been previously absorbed with the lymphocytes used in the experiment^{8,9}. Those who described B lymphocytes as "villous" and "highly" undulate had incubated lymphocytes for 60 min at 0 °C with complement-coated human red blood cells⁷. These conditions can activate B cells and initiate the surface changes described. Although it might seem that these investigators were each describing a different type of cell, the results of the present study would indicate that they were actually describing different stages of activation of B cells. This suggests that conditions for

evaluating peripheral blood lymphocytes as 'T' or 'B' should be standardised.

A study of mouse T and B cells obtained from lymph nodes, incubated in basal salt solution, and fixed before addition of antiserum conjugated to latex spheres by the method of Lo Buglio⁶ agrees with our first report that human T cells have highly villous surfaces while B cells had relatively few microvilli¹⁰. It seems that surface characteristics of unstimulated T and B cells of mice and men are remarkably similar.

I thank Dr T. Makinodan for helpful discussion and criticism, Ned Manti for providing non-plaque forming SV40, Park Trefts for contributing the KLH, Helga Johnson for providing the antisera, and Hideo Naito, Mike Shinohara, and Masauro Tamura for their technical assistance.

MARGUERITE M. B. KAY

Laboratory of Cellular and Comparative Physiology, Gerontology Research Center, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland and Baltimore City Hospitals, Baltimore, Maryland 21224

Received December 24, 1974.

- 1 Nicholson, G. L., Masouredis, S. P., and Singer, S. J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1416 (1971).
- 2 Wolfy, L., et al., *J. exp. Med.*, **140**, 523 (1974).
- 3 Stackpole, C. W., et al., *Science*, **172**, 472 (1971).
- 4 Revel, J. P., in *Scanning Electron Microscopy*, Part III, 542 (ITT Research Institute, Chicago, 1974).
- 5 Molday, R. S., and Dreyer, W. L., *Nature*, **249**, 81 (1974).
- 6 Lo Buglio, A. F., Rinehart, J. J., and Balcerzak, S. P., in *Scanning Electron Microscopy*, Part II, 313 (ITT Research Institute, Chicago 1972).
- 7 Lin, S., Cooper, A. G., and Wortis, H. H., *New Engl. J. Med.*, **289**, 548 (1973).
- 8 Polliack, A., et al., *J. exp. Med.*, **138**, 607 (1973).
- 9 Polliack, A., et al., *J. exp. Med.*, **140**, 146 (1974).
- 10 Kay, M. M. B., et al., *Clin. Immun. Immunopath.*, **2**, 301 (1974).
- 11 Avrameas, S., *Immunochimistry*, **6**, 43 (1969).
- 12 Boyum, A., *J. clin. Lab. Invest.*, **21**, Suppl. 97 (1968).
- 13 Linthicum, D., Sell, S., Wagner, R., and Trefts, P., *Nature*, **252**, 173 (1974).
- 14 Wybran, J., Chantler, S., and Fudenberg, H., *Lancet*, **126** (1973).

Regulation of cell size in fish of tetraploid origin

ALTHOUGH what precisely determines the characteristic size of the cell is still unknown its regulation must have been of enormous significance during evolution^{1,2}. Bigger cells have a relatively lower metabolic rate than smaller ones so that the reduction of cell size is of adaptive value when changing environmental conditions requires an increase in metabolism and *vice versa*^{1,2}. A decrease in cell size, however, must be accompanied by a corresponding loss of DNA if the nucleocytoplasmic relationship, which is fundamental in cell biology, is to be maintained.

We have reported on phylogenetically tetraploid Cyprinid fish, which still keep the tetraploid genome, but exhibit a reduction of cell size, compared with the expected value in tetraploid cells³. This reduction cannot be ascribed to loss of nuclear DNA. We therefore postulated a regulatory mechanism which acts at the level of specific genes. It is known that fish cell size, to some extent, parallels the amount of (28S+18S) ribosomal genes⁴. In a group of closely related plant species the number of ribosomal genes is also reduced with increasing DNA content^{5,6}. Since ribosomes are required for the translation of all RNAs specified by structural genes, a loss of ribosomal genes during the evolution of the tetraploids might well be responsible for a lower synthetic output of the cell, and, in consequence, for the reduction of cell size. In the present investigation we have determined the number of (28S+18S) rRNA genes in the diploid and the phylogenetically tetraploid Cyprinid fish species (Fig. 1).

Surprisingly, a clear-cut 1:2 ratio of the number of (28S+18S) ribosomal genes exists between the diploid and the tetraploid group amounting to a mean number of 272 and 544

Table 1 Cellular parameters in diploid and phylogenetically tetraploid Cyprinid fish species

	<i>Rutilus rutilus</i>	<i>Tinca tinca</i>	<i>Abramis brama</i>	<i>Leuciscus cephalus</i>	<i>Barbus barbus</i>	<i>Cyprinus carpio</i>	<i>Carassius auratus</i>
DNA content per nucleus (% of human leukocytes) ⁷	28	30	36	38	49	50	53
Erythrocyte volume (μm^3)	202.0	203.8	182.0	186.0	190.8	181.6	180.6
Soluble protein content per erythrocyte (pg)	60.1	70.4	63.0	66.7	70.8	64.7	64.9
Nucleolar area (% of nuclear area)	3.7 $\pm$ 0.6*	3.4 $\pm$ 0.3	3.5 $\pm$ 0.6	3.7 $\pm$ 0.4	2.1 $\pm$ 1.0	1.6 $\pm$ 0.4	1.5 $\pm$ 0.2

The erythrocyte volumes were determined with the haematocrit method and the protein content was measured according to Lowry *et al.* (for further details see Schmidtke and Engel⁹). The relative nucleolar size in 20 cells of each species was determined from smears of fin epithelial cells fixed in Carnoy, and stained with acridine orange.

*Mean $\pm$ s.d.

respectively. Although heterologous (28S+18S) rRNA from HeLa cells and not homologous rRNA from fish cells was used, the observed values approximate the true number of ribosomal genes in the Cyprinid fish species very closely. When DNA of *Carassius auratus* was hybridised with homologous (28S+18S) rRNA a saturation value of 0.00054 (rRNA/DNA) was observed⁴; using HeLa rRNA we found a mean saturation value of 0.00052 in this species. These results imply far-reaching homology between fish and human (28S+18S) rRNA cistrons.

It is clear that in the diploid-tetraploid system dealt with in this investigation there is no correlation between cell size and the number of (28S+18S) rRNA genes. It could be assumed, however, that, referring to the expected tetraploid cell size, the reduction to the diploid level was achieved by

diminishing activity of the ribosomal genes during evolution nucleolar size measurements point in this direction (Table 1). The diploid and the tetraploid Cyprinids have nucleoli of very similar size, which suggests similar synthetic rates of rRNA. This is being studied at present.

This work was supported by the Deutsche Forschungsgemeinschaft. The authors thank Dr W. Krone, Ulm, for the gift of labelled rRNA, and Dr K. Bross for advice.

J. SCHMIDTKE
M. T. ZENZES
H. DITTES
W. ENGEL

Institut für Humangenetik und Anthropologie,
Universität Freiburg,
78 Freiburg im Breisgau, Germany

Received November 27, 1974; revised February 21, 1975.

- 1 Szarski, H., *Nature*, **226**, 651-652 (1970).
- 2 Bachmann, K., Goin, O. B., and Goin, G. J., in *Evolution of Genetic Systems* (edit. by Smith, H. H.), 419-450 (Gordon and Breach, New York, 1972).
- 3 Schmidtke, J., and Engel, W., *Biochem. Genet.* (in the press).
- 4 Pedersen, R. A., *J. exp. Zool.*, **177**, 65-78 (1971).
- 5 Siegel, A., Lightfoot, D., Ward, O. G., and Keener, S., *Science*, **179**, 682-683 (1973).
- 6 Maher, E. P., and Fox, D. P., *Nature*, **245**, 170-172 (1973).
- 7 Ohno, S., Wolf, U., and Atkin, N. B., *Hereditas*, **59**, 169-187 (1968).
- 8 Birnstiel, M. L., Sells, B. H., and Purdom, J. F., *J. molec. Biol.*, **63**, 21-39 (1972).
- 9 Bross, K., and Krone, W., *Humangenetik*, **14**, 137-141 (1972).
- 10 Loening, U. E., *J. molec. Biol.*, **38**, 355-365 (1968).
- 11 Bachmann, K., *Chromosoma (Berl.)*, **37**, 85-93 (1972).

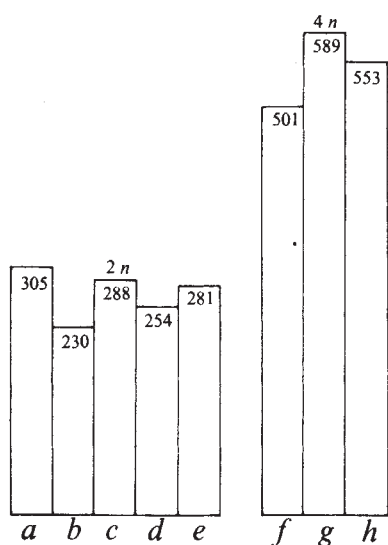


Fig. 1 The number of (28S+18S) rRNA cistrons per diploid genome in diploid and phylogenetically tetraploid Cyprinid fish species; a, *Rutilus rutilus*; b, *Tinca tinca*; c, a natural hybrid from *Rutilus rutilus* and *Abramis brama*; d, *Abramis brama*; e, *Leuciscus cephalus*; f, *Barbus barbus*; g, *Cyprinus carpio*; h, *Carassius auratus*. Each column represents the mean of about five specimens. DNA was isolated from erythrocytes lysed with Triton X-100, followed by treatment with Pronase and a SDS-concentrated saline mixture, several extractions with phenol, in the presence of *m*-cresol and 8-hydroxyquinoline, and chloroform, and precipitation with isopropanol. Alkali-denatured DNA was loaded on Sartorius nitrocellulose membrane filters (35 μg per filter). DNA was hybridised at 65 °C in 6 $\times$ SSC-50% formamide essentially as described by Birnstiel *et al.*⁸ with ³H-labelled (28S+18S) rRNA isolated from ribosomes of HeLa cells as described by Bross and Krone⁹. Specific radioactivity ranged from 40,000 to 50,000 c.p.m. μg^{-1} . Saturating RNA concentration (> 5 $\mu\text{g ml}^{-1}$) and saturating time course (4 h) were determined experimentally. The calculation of the number of 28S and 18S ribosomal genes was based on a molecular weight of fish rRNA of 1.51×10^6 and 0.7×10^6 respectively¹⁰, and a diploid DNA-content of the human genome of 7.3×10^{-12} g (ref. 11).

Abnormal development of preimplantation rat eggs after three days of maternal dietary zinc deficiency

DIETARY deficiency of zinc in pregnant rats causes congenital malformations of high incidence in multiple organ systems^{1,2}. These teratogenic effects of zinc deficiency seem to be related to impairment of nucleic acid synthesis³⁻⁶. We now report that dietary zinc deficiency in the first few days of pregnancy results in abnormal cleavage and blastulation in preimplantation eggs, as early as day 3 of gestation in the rat.

Virgin female Sprague-Dawley rats were mated and fed a purified diet, either complete (100 p.p.m. Zn) or zinc-deficient (< 0.4 p.p.m. Zn), from the beginning of pregnancy. The day of finding sperm in the vaginal smear was considered day 0 of gestation. Diet composition and details of environmental control have been published previously³. Females were chloroformed on day 3 of gestation, the reproductive tract was removed, and the oviducts were isolated and flushed with saline introduced into the fimbriated ostium and collected in a watch glass from the distal end. On day 4 of gestation the uterine horns of anaesthetised females were distended slightly with calcium-free Krebs-Ringer solution containing 0.002% collagenase and 0.002% hyaluronidase introduced by cannula through an incision near the vaginal end. A ligature prevented escape of this fluid. Fifteen minutes later, the reproductive organs were removed to a saline bath, each horn was flushed from the vaginal end through a small incision near the tubo-

Table 1 Preimplantation rat eggs on day 4 of gestation as affected by maternal dietary zinc deprivation

Group	Females No.	Corpora lutea No.	Eggs Flushed		Normal Morula		Blastula		Uncleaved		Abnormal Morula		Blastula	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control (100 p.p.m. Zn)	9	131	101	78*	0	0	99	98†	2	2†	0	0	0	0
Deficient (<0.4 p.p.m. Zn)	12	142	113	80*	5	4†	19	17†	9	8†	34	30†	46	41†

*Embryoblasts recovered
No. of corpora lutea $\times 100$.

†Type of embryoblast
Total no. of embryoblasts $\times 100$.

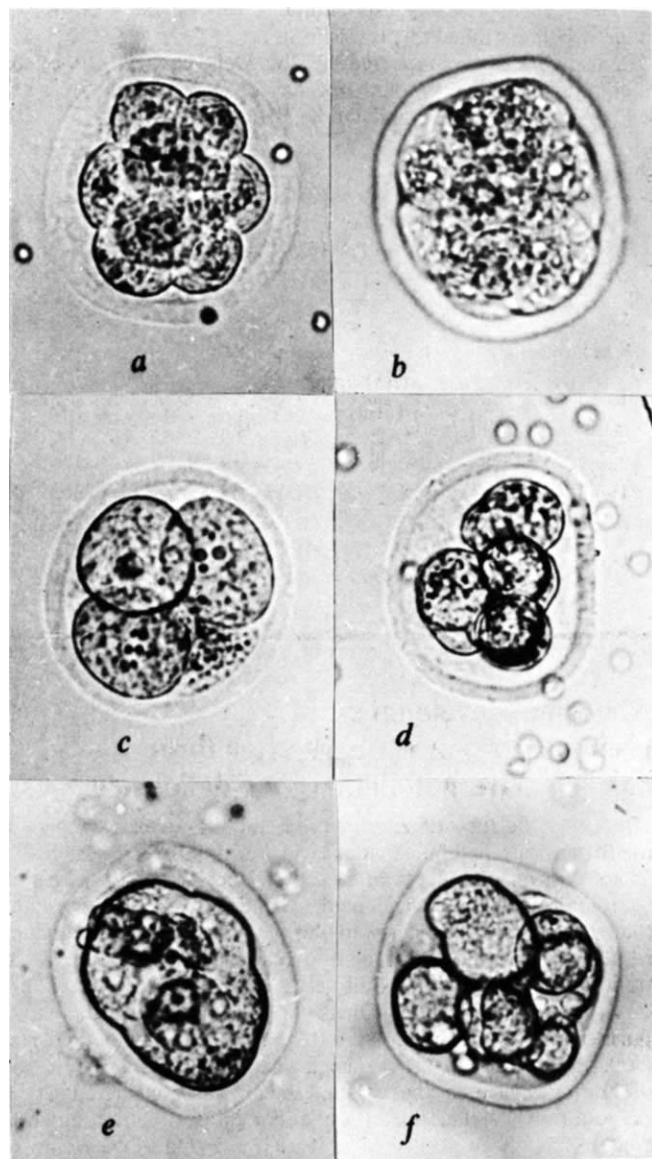


Fig. 1 Examples of embryos obtained from the oviducts of control (*a* and *b*) and zinc deprived (*c-f*) female rats on day 3 of gestation. Note the presence of an intact zona pellucida in all examples. All embryos of both control and deficient dams were stained successfully with neutral red following photography. *a*, Control, eight-cell stage; *b*, control, sixteen-cell stage; *c*, abnormal embryo with three large intact blastomeres and the cellular debris of a fourth; *d*, abnormal seven-cell embryo containing one excessively large blastomere; *e*, abnormal embryo, cell boundaries lacking between blastomeres giving the appearance of a syncytium; *f*, abnormal embryo, showing derangement of orientation and great variation in size of blastomeres.

uterine junction, and the flushings were collected in watch glasses. The procedure yielded approximately 80% of the anticipated embryos, as based on correlation of the number of recovered eggs with the number of corpora lutea in the corresponding ovary (Table 1).

Cleaving eggs obtained by this method responded normally to supravital staining, retained the zona pellucida for more than 7 h in normal saline at pH 7.4⁷, and seemed to be identical morphologically to a small number of samples obtained from deficient and control animals by the methods of Nicholas⁸. Specimens were examined and photographed in saline.

In tubal flushings from seven control rats on day 3 of gestation, all eggs were in the eight or 16 cell stages (Fig. 1 *a* and *b*). In 11 zinc-deficient rats, 68% of the eggs on day 3 of gestation were in the eight-cell stage and appeared to be normal. However, the remaining eggs had either failed to cleave and were undergoing necrosis with disruption and fragmentation of the cellular contents, or consisted of three to seven blastomeres lacking normal orientation and showing variation in size (Fig. 1 *c-f*).

On day 4 of gestation, only two of 101 eggs recovered from control females had failed to undergo cleavage, while all remaining eggs were in the blastocyst stage (Table 1 and Fig. 2 *a* and *b*). Eggs from three of 12 zinc-deficient females were normal morulae or blastulae; all remaining preimplantation embryos from zinc deficient females were abnormal (Table 1 and Fig. 2 *c-f*).

These results clearly show that dietary zinc deficiency in the pregnant rat rapidly produced abnormal development of preimplantation embryos. This is the first time to our knowledge that abnormal development of cleaving eggs has been shown to result from a nutritional deficiency of the maternal diet. It is generally thought that embryos are insensitive to environmental influences before implantation and that exogenous teratogens are either lethal or without lasting effect during this period^{9,10}. In the case of zinc deficiency in rats given a deficient diet only from day 0 to day 5 of gestation, preimplantation losses were minimal, and foetal malformations at term were only slightly increased over those of controls². Thus it is possible that the morphologically abnormal blastocysts described here may recover to develop normally but this question remains to be answered.

The uterine fluid of rabbits has recently been found to contain significant amounts of zinc, approximately twice the concentration of the free-lying blastocysts¹¹. Uptake of zinc by uterine fluid was, however, slow as compared with that of other reproductive tract components such as the endometrium¹¹. It is therefore possible that with a maternal dietary deficiency of zinc, the milieu of the conceptus (the oviductal and uterine fluids) becomes inadequate in zinc as a consequence of competition with tissues having higher avidity for available zinc supplies in the face of the low maternal plasma zinc levels, which are known to occur¹². The finding of abnormalities in cleaving eggs on day 3 of gestation suggests that intraluminal zinc may be reduced to critical levels before this time under these conditions.

Recent work has shown that cleaving mammalian eggs

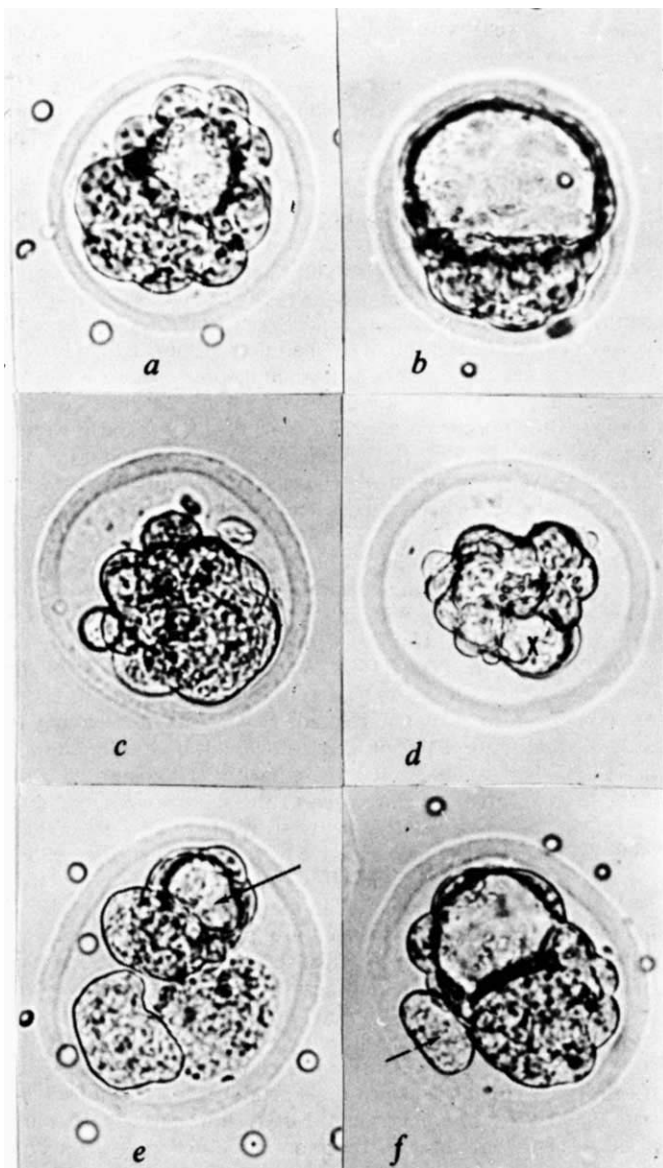


Fig. 2 Examples of embryos obtained from the uterine horns of control (*a* and *b*) and zinc deprived (*c*–*f*) female rats on day 4 of gestation. Note the presence of an intact zona pellucida in all examples. All embryos of both control and deficient dams were stained successfully with neutral red following photography. *a*, Control, early blastula stage; *b*, control, normal blastula; *c*, abnormal morula showing variation in blastomere size; *d*, early, blastocyst from zinc deficient female showing derangement of blastomeres and beginning of blastocoel (×); *e*, abnormal blastulation with dissociated blastomeres and a degenerating mass of cytoplasm. Blastocoel indicated by arrow; *f*, abnormal, lateral continuation of blastocoel cavity (arrow).

incorporated labelled precursors of DNA and RNA¹³ and that mitotic inhibitors or compounds which suppress RNA synthesis adversely affected blastogenesis^{14–16}. Thus, the abnormal development of preimplantation eggs reported here may result from impaired synthesis of nucleic acids, a zinc-requiring process.

This work was supported in part by a research grant from the National Institute of Child Health and Human Development.

LUCILLE S. HURLEY
RUTH E. SHRADER

Department of Nutrition,
University of California,
Davis, California 95616

Received December 23, 1974.

- Hurley, L. S., and Swenerton, H., *Proc. Soc. exp. Biol. Med.*, **123**, 692–696 (1966).
- Hurley, L. S., Gowan, J., and Swenerton, H., *Teratology*, **4**, 199–204 (1971).
- Swenerton, H., Shrader, R., and Hurley, L. S., *Science*, **166**, 1014–1015 (1969).
- Sandstead, H. W., and Rinaldi, R. A., *J. Cell Physiol.*, **73**, 81 (1969).
- Terhune, M. W., and Sandstead, H. W., *Science*, **117**, 68–70 (1972).
- Chesters, J. K., *Trace element metabolism in animals*, **2**, 39–51 (University Park Press, Baltimore, 1974).
- Alloiteau, J. J., and Psychoyos, A., *C.r. hébd. Seanc. Acad. Sci., Paris*, **262**, 1561–1564 (1966).
- Nicholas, J. S., *The rat in laboratory investigation*, 51–67 (Hafner, New York, 1942).
- Wilson, J. G., *Environment and birth defects*, **16**, 135 (Academic, New York, 1973).
- Saxen, L., and Rapola, J., *Congenital defects*, 112–118 (Holt, Rinehart and Winston, New York, 1969).
- McIntosh, J. E. A., and Lutwak-Mann, C., *Biochem. J.*, **126**, 869–876 (1972).
- Dreosti, I. E., Tao, S., and Hurley, L. S., *Proc. Soc. exp. Biol. Med.*, **128**, 169–174 (1968).
- Graham, C. F., *The cell cycle in development and differentiation*, 293–310 (Cambridge University Press, 1973).
- Wilson, J. G., *Harper Hosp. Bull.*, **24**, 109–118 (1966).
- Snow, M. H. L., *The cell cycle in development and differentiation*, 87–102 (Cambridge University Press, 1973).
- Adams, C. E., Hay, M. F., and Lutwak-Mann, C., *J. Embryol. exp. Morphol.*, **9**, 468–491 (1961).

Temperature-sensitive expression of differentiation in transformed myoblasts

NUMEROUS studies have dealt with the replication of tumour viruses in fibroblasts, but little is known about the mechanisms by which such viruses interfere with the control of specific functions in fibroblasts or in differentiating cells. It is known that tumour viruses can transform cells from differentiated tissues^{1–7}. Chick embryo myoblasts can be transformed by Rous sarcoma virus (RSV), (refs 2 and 3); transformation, or at least infection, prevents neither formation of myotubes nor the biochemical changes which ordinarily follow myoblast fusion⁸; Easton and Reich did not, however, purify infected cells by serial passage. We report the *in vitro* transformation of chick embryo myogenic cells with a temperature-sensitive mutant of RSV. We show that transformed myoblasts, after serial passage, lose their ability to differentiate at the permissive temperature (36 °C) but will differentiate to form myotubes—if transformation is blocked by a shift to the non-permissive temperature (41 °C).

Chick myoblasts derived from thigh muscles of 11-d-old chick embryos were infected with the Schmidt-Ruppin ts68 strain of RSV (ref. 9) and incubated at 36 °C (permissive temperature). On day 2 after infection, the presence of myotubes could be detected in all the Petri dishes with no obvious differences between mock-infected cultures and RSV-infected cultures. On day 3 after infection, there was still no difference between the mock-infected cultures and RSV-infected cultures. On day 4 after infection, some clumps of rounded cells began to appear in the infected cultures.

In the mock-infected chick embryo myoblasts (Fig. 1*a*) after 5 d in culture, most of the mononucleated cells have been replaced by long myotubes, each containing numerous nuclei. There are also a number of mononucleated cells which are believed to be fibroblasts. These cells form a monolayer and are never found in clumps or large aggregates. In the RSV-infected cultures after 5 d (Fig. 1*b*) the number of myotubes in the plate is reduced and many aggregates of rounded cells are found; most of these are attached to myotubes. This situation is in good agreement with results described previously^{9,8}, although it is not clear whether all the rounded cells are transformed cells or clumps of dead cells. It is likely that these rounded cells are indeed transformed cells, since no such cells ever appeared in RSV-infected cultures maintained at 41 °C (Fig. 1*c*). We also found, as did Easton and Reich⁸, that infected myoblasts will form myotubes and that these myotubes are not stable and will disappear on further incubation.

The clumps of rounded cells (Fig. 1*b*) were allowed to grow for a few more days, then dispersed with trypsin and distributed into new Petri dishes. The medium was changed to a mixture of Dulbecco's modified Eagle's MEM and Medium 199 (5:2); 10% foetal calf serum and 1% chick embryonic extract were also

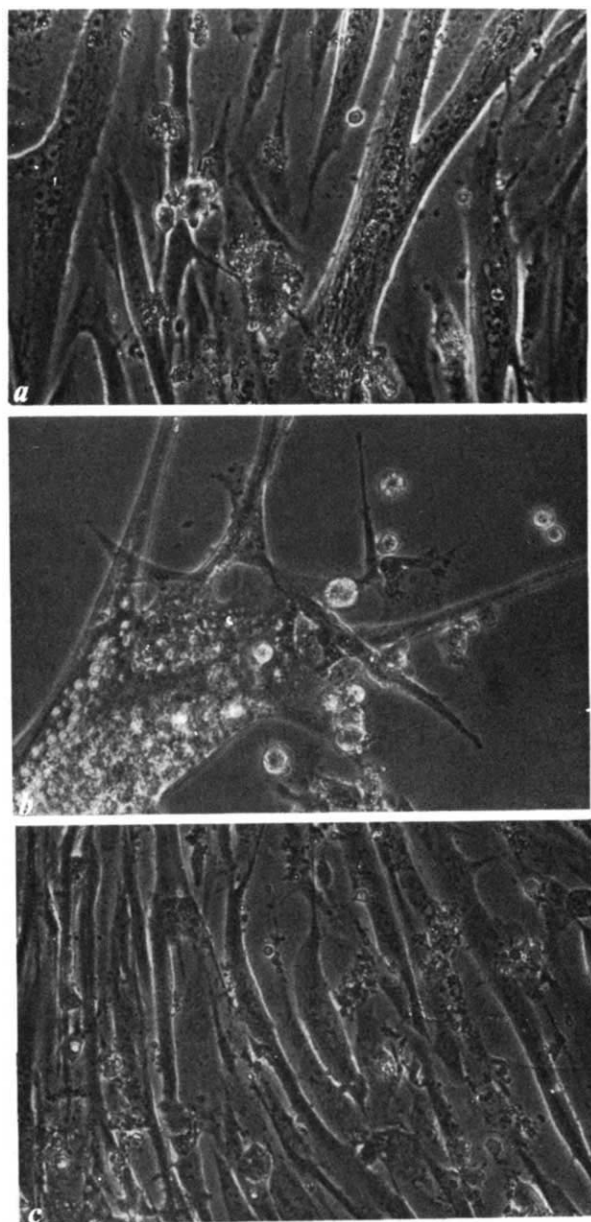


Fig. 1 Phase contrast photographs of chick embryo myoblasts infected with RSV *ts68*. *a*, Mock-infected culture on day 5 after infection. *b*, Infected culture maintained for 5 d at 36 °C; clumped cells have appeared. *c*, Infected culture maintained for 5 d at 41 °C. Chick embryo myoblasts were prepared by a modification of Konigsberg's method¹⁶. Thigh muscles from 11 day embryos, dissected free of skin and bones, were minced with scissors and incubated at 37 °C in phosphate-buffered saline (PBS): NaCl 137 mM, KCl 2.6 mM, Na₂HPO₄ 8.1 mM, KH₂PO₄ 1.4 mM for 15 min. The fragments were dispersed by pipetting through a Pasteur pipette and the dispersed cell suspension was centrifuged at 500*g* for 1 min. The upper nine-tenths of the supernatant was removed, diluted with complete medium (Dulbecco's modified Eagle's MEM containing 10% horse serum and 2% chick embryonic extract) and preplated at 5–10 × 10⁶ cells on gelatin-coated Falcon tissue-culture Petri dishes (100 mm) to remove fibroblasts. (This procedure usually yields a 90% or better pure myoblast population.) After 15–20 min at 37 °C in a 5% CO₂ incubator, the unattached cells were transferred to gelatin-coated tissue culture Petri dishes (100 mm) at a plating density of 2 × 10⁶ cells and the plates were incubated at 37 °C. Five hours later the cells were washed once with PBS and infected with 2 ml of RSV *ts68* containing 10⁶ focus-forming units ml⁻¹. After adsorption for 60 min at 36 °C, the cells were overlaid with complete growth medium and incubated at 36 °C in 5% CO₂ in air. Many multinucleated myotubes can be seen.

present. The cells were serially passed with a starting density of 5 × 10⁵ cells per tissue-culture Petri dish (100 mm) and trypsinised when the plates reached confluency (about 8–10 × 10⁶ cells per plate). During these manipulations, one of the dishes was accidentally left too long and became overcrowded. The sheet of cells detached from the dish and the remaining cells were overlaid with fresh medium. A few clones had grown after a further 10 d; three of them (cl-1, cl-2, cl-3) were picked and further maintained in culture. After about ten generations, one Petri dish of each of the three clones and one Petri dish of the uncloned parental culture were shifted to the non-permissive temperature (41 °C). A change in the morphology of the cells was evident, 24 h after the shift, in all Petri dishes. The cells were more flattened and more dispersed, and some cells had lost the spindle shape characteristic at 36 °C. Figure 2*a* illustrates the changes which occurred after 3 d at 41 °C in both the uncloned parental culture and in cl-3. Numerous myotubes could be seen among a layer of mononucleated cells. In cl-1 and cl-2, no such myotubes could be seen even after a longer period of time at 41 °C.

On the other hand, at 36 °C, no formation of myotubes could be observed in any of the cultures. A more quantitative study, using the parental uncloned culture, is presented in Table 1. It is clear that after the shift to 41 °C there is a change in the overall composition of the population. Whereas at 36 °C, 99% of the cells remain mononucleated, after the shift up to 41 °C, 60% of the cells become polynucleated. It is, however, striking to observe that only 12% of these polynucleated cells contain more than ten nuclei as compared with 71% when primary chick embryo myoblasts are used (Table 1). This was taken to reflect the fact that the RSV-transformed myoblast population contains fewer cells which are able to fuse than do the primary myoblast populations. From this experiment it is clear that RSV-transformed myoblasts can be isolated and that these myoblasts, after serial passage, are prevented from further differentiation. Nevertheless this function is not lost, since following a shift up to the non-permissive temperature they will regain this function and will differentiate to form myotubes.

The nature of the cells which do not fuse at 41 °C remains to be elucidated. The most likely explanation is that these are transformed fibroblasts since the starting primary culture consists of 90–95% myoblasts and 5–10% fibroblasts. One must also consider that some of the mononucleated cells are transformed myoblasts which have definitively lost their ability to differentiate since clones of myogenic cells which do not fuse have already been described¹² (N. Teng, unpublished). A clear demonstration of either one or both of these hypotheses will require the isolation of morphologically pure clones of cells.

Finally, there is a striking difference between the myotubes which appeared after infection and the ones which were formed after the shift to 41 °C, since the latter are very stable and remain on the dish as long as would myotubes in an uninfected culture.

Fig. 2 Morphology of transformed myoblasts at 41 °C. Photomicrograph of the uncloned parental culture maintained for 3 d at 41 °C.

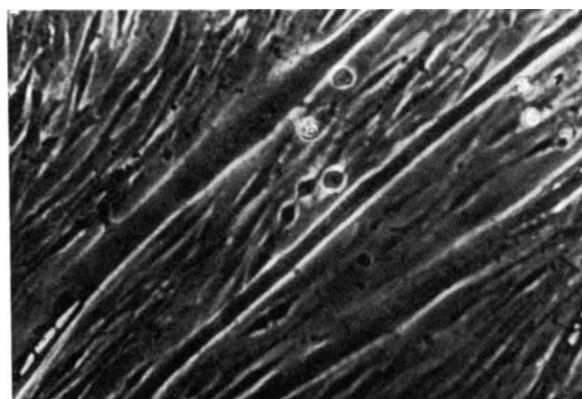


Table 1 Myotube formation by RSV-transformed myoblasts at 36 and 41 °C

	% Mono-nucleated cells	% Polynucleated cells 2-3 nuclei	4-10 nuclei	> 10 nuclei
RSV-transformed myoblasts at 36 °C	99	1		
RSV-transformed myoblasts at 41 °C	40	12	36	12
Primary myoblasts at 36 °C	8	3	18	71

RSV-transformed myoblasts were seeded at 36 or 41 °C Primary chick embryo myoblasts, prepared as described in the legend to Fig. 1a, were incubated at 36 °C. Four days after seeding, one Petri dish of each culture was fixed and stained with haematoxylin. (A minimum of 500 nuclei were counted in each plate.)

In conclusion, we have shown that it is possible to isolate transformed chick embryo myoblasts in which the expression of differentiation is under the control of a viral gene. It is too soon to make any speculation about the mechanism of control, especially since nothing is known as yet about the other changes which usually follow the event of fusion such as myosin synthesis¹¹, appearance of cholinergic receptors^{12,13} and regulation of various enzymes^{14,15}.

This work was supported by grants from the NIH to Dr V. M. Ingram and to Dr P. W. Robbins. M.Y.F. holds an EMBO fellowship.

MARC Y. FIZSMAN
PINHAS FUCHS*

Department of Biology and Center for Cancer Research,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received January 3; revised February 17, 1975.

* Present address: Department of Biophysics, Israel Institute for Biological Research, PO Box 19, Ness Ziona, Israel.

- Ephrussi, B., and Temin, H. M., *Virology*, **11**, 547-552 (1960).
- Kaighn, M. E., Ebert, J. D., and Stott, P. M., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 133-140 (1966).
- Lee, H. H., Kaighn, M. E., and Ebert, J. D., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 521-525 (1966).
- Fogel, M., and Defendi, V., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 967-973 (1967).
- Lee, H. H., Kaighn, M. E., and Ebert, J. D., *Int. J. Cancer*, **3**, 126-136 (1968).
- Plessac, B., and Calothy, G., *Science*, **185**, 709-710 (1974).
- De Vitry, F., et al., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3575-3579 (1974).
- Easton, T. G., and Reich, E., *J. biol. Chem.*, **247**, 6420-6431 (1972).
- Kawai, S., and Hanafusa, H., *Virology*, **46**, 470-479 (1971).
- Traikas, H., and Schubert, D., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2377-2381 (1974).
- Paterson, B., and Strohmman, R. C., *Devl Biol.*, **29**, 113-138 (1972).
- Fambrough, D., and Rash, J. E., *Devl Biol.*, **26**, 56-68 (1971).
- Patrick, J., Heinemann, S. F., Lindstrom, J., Schubert, D., and Steinbach, J. H., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2762-2766 (1972).
- Coleman, J. R., and Coleman, A. W., *J. Cell Physiol.*, **72**, suppl. 1, 19-34 (1968).
- Shainberg, A., Yagil, G., and Yaffe, D., *Devl Biol.*, **25**, 1-29 (1971).
- Konigsberg, J. R., McElvain, N., Tootle, M., and Herrmann, H., *J. biophys. Biochem. Cytol.*, **8**, 333-343 (1960).

Diffusible mating-type factors induce macrocyst development in *Dictyostelium discoideum*

In cellular slime moulds the formation of multicellular macrocysts constitutes an alternative pathway of development to that of fruiting bodies. In appropriate conditions macrocysts form in certain strains of *Dictyostelium* and *Polysphondylium*¹. The existence of mating types in cellular slime moulds was suggested when certain combinations of amoebae of different strains resulted in macrocyst development^{2,3}. Since the aggregation of cells which leads to fruiting body formation in *D. discoideum* is mediated by cyclic AMP^{4,5}, we decided to investigate whether the interaction between mating types is also mediated by a small, diffusible molecule. Our results indicate that such a diffusible

mating factor does indeed regulate macrocyst formation in *D. discoideum*.

Stock cultures of two complementary, haploid mating types of *D. discoideum* (strains NC-4 and V-12, given by Dr D. W. Francis) were maintained on *Escherichia coli* (strain B/r) on 0.2% Cerophyl agar. These strains can form macrocysts when mixed but not when cultured separately³. The general macrocyst-forming conditions of Nickerson and Raper were used in all experiments.⁷

In the first set of experiments, a suspension (5.0 ml) of bacteria and spores of one strain of *D. discoideum* in distilled water was pipetted into sterile dialysis tubing (pore size 4.8 nm). The tubing with ends tied was placed in a culture plate. An equal volume of spore suspension of the opposite strain (experimental) or the same strain (control) was distributed uniformly over the tubing and the agar. The plates were stored in light-tight metal canisters at 23 ± 1 °C. In every experiment separate cultures of V-12 and NC-4, which never produced macrocysts, and a mixture of V-12 and NC-4, which always produced macrocysts, were run as controls. Macrocyst formation was assessed by microscopic (× 25-50) examination of plates after 7 and 14 d. Whenever macrocysts formed they conformed in structure to that described before^{1,2,4,7}.

Table 1 summarises the results of these experiments. When NC-4 was placed in the dialysis tubing and V-12 was outside, macrocyst formation was induced in eight out of 21 cultures. Many macrocysts formed along the outside of the dialysis membrane, but few formed further than a few centimeters from the tubing. The reverse combination, with V-12 inside the tubing and NC-4 outside, yielded no macrocysts. Under the conditions used either macrocysts developed fully and were evident after 7 or 14 d, or no development began. Small numbers of fruiting bodies appeared on all culture plates. Thus cell contact between opposite mating types of *D. discoideum* is not essential for macrocyst formation.

Table 1 Induction of macrocysts across a dialysis membrane

Experimental situation	No. of cultures	No. of cultures with macrocysts
		NC-4 V-12
NC-4 alone	9	0 NA
V-12 alone	9	NA 0
(NC-4) × NC-4	7	0 NA
(NC-4) × V-12	21	0 8
(V-12) × NC-4	18	0 0

When dialysis tubing was used the cells contained within the tubing are listed first within brackets. NA, not applicable.

A second series of experiments was carried out to determine whether the continual presence of the opposite mating type was essential for macrocyst induction. Each strain was cultured separately in macrocyst-forming conditions. After 18 h, the cells were dislodged from the agar surface with a bent glass rod and the suspension was centrifuged at 2,000g for 5 min to pellet the whole cells. The supernatant was filtered through a sterile Millipore filter and the final sterile, cell-free supernatant (conditioned medium) was poured over a fresh culture (0 h culture) of the opposite strain. After 7 or 14 d, cultures were examined for the presence of macrocysts. For controls, the supernatant of one strain was added to cells of the same strain and distilled water instead of supernatant was added to individual cultures of each strain.

Table 2 shows that NC-4 supernatant induced macrocyst formation in V-12 66% of the time, but the reverse combination never resulted in macrocyst formation. These data agree with the results of the first set of experiments and emphasise that cell contact between mating types is not essential for complete macrocyst formation. Furthermore, the data indicate that a stable inducing factor (molecular weight about 12,000) was released by NC-4 very early under macrocyst-forming conditions. The fact that V-12 supernatant did not induce a response in NC-4 cells suggests that this factor was unstable or possibly non-

Table 2 Effect of extracellular medium on macrocyst induction

Experimental situation	No. of cultures	No. of cultures with macrocysts
NC-4 supernatant to NC-4 cells	6	0
V-12 supernatant to V-12 cells	4	0
NC-4 supernatant to V-12 cells	35	22
V-12 supernatant to NC-4 cells	25	0
Distilled water to NC-4 cells	13	0
Distilled water to V-12 cells	13	0

existent. The ability easily to induce V-12 to form macrocysts with NC-4 supernatant provides an excellent bioassay system for use in the purification of the mating factor.

Macrocyst development might be divided into two events: aggregation of single cells to form multicellular clumps, followed by differentiation of the aggregates into mature macrocysts. It is unlikely that cyclic AMP is the small molecule that is involved in the formation of macrocysts of *D. discoideum*, for fruiting bodies appeared in isolated cultures of NC-4 and V-12 when macrocysts could not form. Furthermore, it seems that once macrocyst aggregation is induced, the events of differentiation must also be triggered since macrocyst development always went to completion.

Although our results suggest that one strain (NC-4) could be an initiator or dominant strain in a sequential induction system, it is possible that the inducing factor from the opposite mating type (V-12) is simply less stable under our conditions.

This research was supported in part by a grant from the National Research Council of Canada.

DANTON H. O'DAY
KEITH E. LEWIS

Department of Zoology and Erindale College,
University of Toronto, Mississauga,
Ontario, Canada L5L 1C6

Received December 3, 1974; revised February 14, 1975.

¹ Blaskovics, J. C., and Raper, K. B., *Biol. Bull.*, **113**, 58-88 (1957).

² Clark, M. A., Francis, D., and Eisenberg, R., *Biochem. biophys. Res. Commun.*, **52**, 672-678 (1973).

³ Erdos, G. W., Raper, K. B., and Vogen, L. K., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1828-1830 (1973).

⁴ Filosa, M. F., and Dengler, R. E., *Devl Biol.*, **29**, 1-16 (1972).

⁵ Bonner, J. T., *A. Rev. Microbiol.*, **25**, 75-92 (1971).

⁶ Barkley, D. S., *Science*, **165**, 1133-1134 (1969).

⁷ Nickerson, A. W., and Raper, K. B., *Am. J. Bot.*, **60**, 190-197 (1973).

Effects of progesterone and EDTA on cyclic AMP and phosphodiesterase in *Dictyostelium discoideum*

WHEN *Dictyostelium discoideum* amoebae are nutritionally deprived, they undergo a developmental programme which results in the formation of fruiting bodies composed of spore and stalk cells. Early events in this cycle are marked by the aggregation of previously individual amoebae. During this process they become elongated, and establish specific intercellular contacts, resulting in the formation of cell streams¹. Orientation of cell movement during this period is thought to be directed by an external gradient of cyclic AMP (ref. 2). This compound elicits a chemotactic response from starved cells but not from growing cells^{3,4} and can induce a period of coordinated movement in nutritionally deprived cells⁵. A phosphodiesterase is also excreted by aggregating cells^{6,7} activity of which modulates the cyclic AMP gradient. Thus, a general model for communication between cells during aggregation is available. Little is known, however, about the regulation of metabolic events which result in the differentiation of growth phase cells into aggregation-competent, cyclic AMP-sensitive cells, and in particular, the influence of cyclic AMP on these processes. Since in other eukaryotes, changes in internal cyclic AMP are thought to mediate the actions of a variety of hormones, and regulate cell growth and

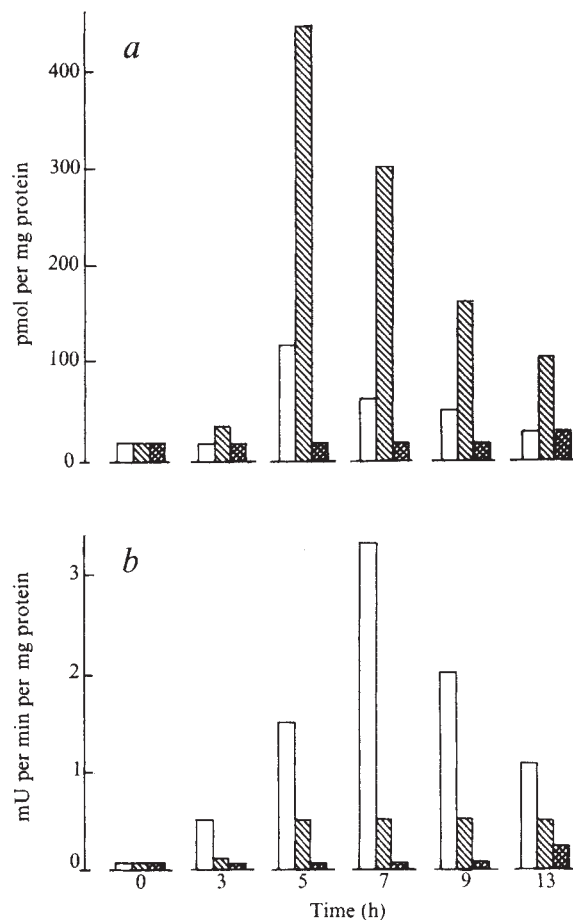


Fig. 1 Intracellular cyclic AMP (a) and phosphodiesterase (b) levels (which includes both cytoplasmic and membrane-bound activities) in *D. discoideum* cells incubated at 22 °C in buffer (open columns), buffer plus 10^{-3} M EDTA (hatched columns), buffer plus 3×10^{-5} M progesterone (cross-hatched columns). 1.4×10^7 Ax-2 amoebae, an axenic haploid strain⁸, were plated on to 100 $\times$ 20 mm Falcon tissue culture dishes in 5 ml mM buffer [5 mM 2-(N-morpholino)-ethane sulphonic acid, pH 6.2, 10 mM KCl, 5 mM MgCl₂] with or without EDTA or progesterone⁸. For studies in which cyclic AMP was measured, the media from four plates was aspirated at the indicated times and cells were immediately harvested in 5% cold TCA. Samples were further prepared by the procedure of Maganillo and Vaughan¹¹, except that Dowex chromatography was performed at pH 6. Aliquots were assayed at three dilutions for cyclic AMP using the method of Gilman¹². For measurement of phosphodiesterase activity, cells were harvested in their media, centrifuged at 3,000g for 2 min and washed once with 50 mM Tris, pH 8, 4 mM MgCl₂. The pellet was frozen, then thawed in 1 ml 50 mM Tris, pH 8, 4 mM MgCl₂ and dialysed overnight against the same buffer. Aliquots were assayed at 30 °C using the method of Brooker¹². Protein determinations were performed according to Lowry *et al.*¹³. Values represent the average of four experiments.

development, we investigated the possible intracellular role of this nucleotide in *D. discoideum*.

It is generally agreed that abnormal aggregation occurs if amoebae produce an improper chemotactic signal. If changes in intracellular cyclic AMP affect processes critical to cell differentiation, however, it is also possible that abnormal aggregation occurs because cells have not been 'induced' to respond properly to a signal. A recent study of the effects of various compounds on *D. discoideum* revealed that cells treated with either EDTA or progesterone aggregate abnormally⁸. In the presence of 10^{-3} M EDTA, a concentration below that of the magnesium ion concentration in the media, cells undergo extensive clumping and very limited streaming. This aberrant aggregation is completed within the normal period of time, 9 h. In contrast, 3×10^{-5} M progesterone causes cells to

remain dissociated and not exhibit any of the morphological changes observed during starvation, even after 18 h incubation. When the steroid is removed from the media, cells proceed to aggregate normally. Since these two compounds inhibit cell aggregation in markedly different ways, we were interested in determining the level(s) at which they act. Here, we report their effects on both intracellular and extracellular cyclic AMP and phosphodiesterase and relate these findings to cell morphology.

Figure 1 shows that the intracellular cyclic AMP concentration of amoebae increases by six to eightfold approximately 5 h after transfer on to Petri dishes in non-nutritive media. At this time, cells become elongated and establish specific cell-cell contacts typical of the early aggregation phase. Similar qualitative changes in cyclic AMP levels in amoebae developing on Millipore filters have been reported¹⁴. The increase in cyclic AMP content is followed by an eight to tenfold induction of phosphodiesterase activity. The kinetics of enzymic stimulation correlate well with the decrease in cellular cyclic AMP observed after 7 h. Addition of 10^{-3} M EDTA, which enhances the formation of small cell aggregates, produces over a 40-fold stimulation of the cellular cyclic AMP content. Even after 11 h, when the cyclic AMP concentration of untreated cells has returned to almost the basal level, that of EDTA-treated cells

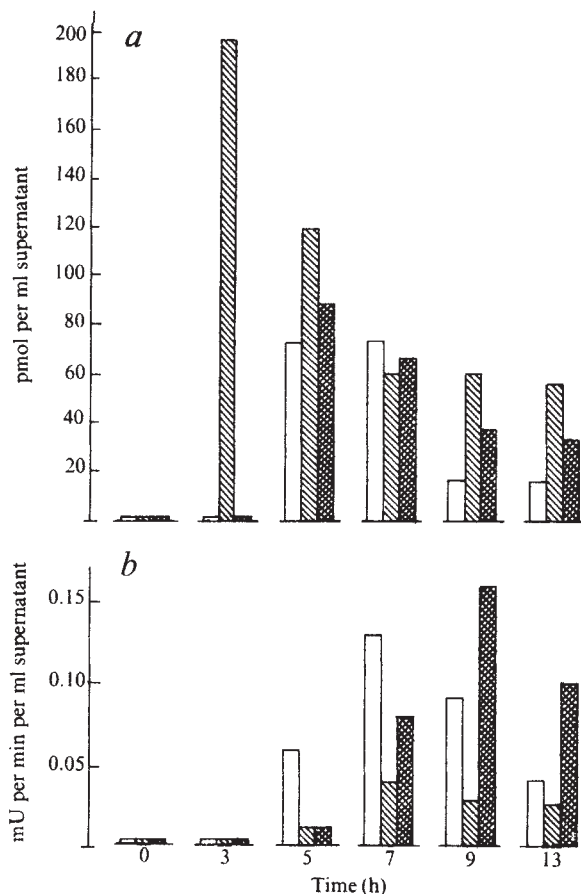
is still greatly elevated. This prolonged elevation can be explained by the fact that no increase in phosphodiesterase activity is observed under such conditions. Treatment with 3×10^{-5} M progesterone, which results in cells retaining their growth-phase appearance, inhibits both the rise in cyclic AMP and phosphodiesterase activity.

The kinetics of cyclic AMP excretion are shown in Fig. 2. In the case of untreated amoebae, the concentration of nucleotide in the media increases after 5–6 h of starvation and then decreases. This decline is associated with a corresponding increase in an extracellular phosphodiesterase. Cells incubated with 10^{-3} M EDTA excrete a large excess of cyclic AMP and do so several hours earlier than control cells. During the next 12 h there is a gradual decline in the cyclic AMP level but not a return to that of control cells. The small increase in extracellular phosphodiesterase activity seen in EDTA-treated cells is probably responsible for this gradual decrease. It therefore seems that EDTA increases the levels of cyclic AMP by inhibiting its intracellular and extracellular degradation. Since the extracellular nucleotide is detected early, when cells are incubated with EDTA, it is also possible that synthesis of the nucleotide is stimulated under these conditions. Surprisingly, progesterone does not inhibit the appearance of either cyclic AMP or phosphodiesterase in the media, and may, in fact, enhance this process. The contrasting effects of progesterone on the internal and external cyclic AMP and phosphodiesterase patterns may be explained by the existence of two systems, only one of which (intracellular) is progesterone sensitive. A simpler explanation, however, would be that progesterone stimulates the cellular excretion of both cyclic AMP and phosphodiesterase. Other experiments (C. K. and P. B., unpublished) tend to support this model.

The variations in the extracellular cyclic nucleotide concentrations are basically unchanged when cells are incubated with steroid. Since the changes in extracellular cyclic AMP concentrations occurring in a cell population probably reflect the changes which occur at the individual cell level, this result suggests that the chemotactic signal, thought to orientate cell movement, is unaffected. Progesterone probably prevents amoebae from responding to, rather than producing, the chemotactic signal. This insensitivity to the chemotactic signal may be directly related to the inability of cells to accumulate cyclic AMP in the presence of steroid. The 'inactivated' amoebae therefore tend to remain as isolated cells. This interpretation of the intracellular role of cyclic AMP is compatible with the morphological changes seen in the presence of EDTA, that is, the formation of numerous small aggregates. EDTA, which greatly increases the cellular levels of cyclic AMP, would produce highly 'activated' amoebae, metabolically prepared to respond to a proper orienting signal. Measurements of extracellular cyclic AMP and phosphodiesterase suggest that these amoebae do not produce a proper chemotactic signal. They do not stream normally, but instead form small aggregation centres. This model predicts that high concentrations of exogenously added cyclic AMP (which would both accumulate within the cells and perturb the extracellular signalling pattern) should mimic the morphological effects seen with EDTA. We have found that cells incubated in our conditions with 2×10^{-4} M cyclic AMP form many aggregates without visible streaming (C. K. and P. B., unpublished). Chemotactic assays⁴ used as an independent measure of cell differentiation, show also that progesterone-treated cells resemble growth-phase amoebae, whereas EDTA-treated cells behave as differentiated amoebae (C. K. and P. B., unpublished). Obviously, a more extensive analysis must be undertaken to determine the specificity of action of progesterone and EDTA on *D. discoideum*. These experiments, which include determining their effects on adenylyl cyclase, general RNA and protein synthesis, and cellular excretion, are in progress and should help clarify the processes involved in cell aggregation and the levels at which cyclic AMP influences this phenomena.

We thank Dr R. Kram (Brussels) for supplying cyclic AMP binding protein. C. K. is an American Cancer postdoctoral

Fig. 2 Cyclic AMP (a) and phosphodiesterase (b) excreted during starvation by control (open columns), EDTA- (hatched columns) and progesterone-treated (cross-hatched columns) cells. Amoebae were incubated as described in the legend to Fig. 1. At the indicated times, the media which was aspirated from the cells used for intracellular determinations was either made 1% in TCA and prepared for cyclic AMP assays or dialysed for 5 h against 5 mM Tris, pH 8, 4 mM MgCl_2 , and then overnight against 50 mM Tris, pH 8, 4 mM MgCl_2 . The latter samples were then assayed for phosphodiesterase activity. Values represent the average of four experiments.



fellow. This research was supported by the Fondation pour la Recherche Medicale Francaise.

C. KLEIN
P. BRACHET

Département de Biologie Moléculaire,
Unité de Génétique Moléculaire,
Institut Pasteur,
25 rue du Dr Roux, 75015 Paris, France

Received November 19, 1974; revised January 20, 1975.

- ¹ Beug, H., Gerish, G., Kempff, S., Riedel, V., and Cremer, G., *Expl. Cell. Res.*, **63**, 147-158 (1970).
- ² Konijn, T. M., *J. Bact.*, **99**, 503-509 (1969).
- ³ Konijn, T. M., van de Meene, J. G. C., Bonner, J. T., and Barkley, D. S., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1152-1154 (1967).
- ⁴ Bonner, G. T., et al., *Dev. Biol.*, **20**, 72-87 (1969).
- ⁵ Robertson, A., Drage, D. G., and Cohen, M. H., *Science*, **175**, 333-335 (1972).
- ⁶ Pannbacker, R. G., and Bravard, L. J., *Science*, **175**, 1014-1015 (1972).
- ⁷ Riedel, V., and Gerish, G., *Biochem. biophys. Res. Commun.*, **42**, 119-124 (1971).
- ⁸ Brachet, P., and Klein, C., *Expl. Cell. Res.* (in the press).
- ⁹ Watts, D. T., and Ashworth, J. M., *Biochem. J.*, **119**, 171-174 (1970).
- ¹⁰ Maganillo, V., and Vaughan, M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 269-273 (1972).
- ¹¹ Gilman, A. G., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 305-312 (1970).
- ¹² Brooker, G., Thomas, L. J., and Appleman, M. M., *Biochemistry*, **7**, 4177-4179 (1968).
- ¹³ Lowry, O. H., Rosebrough, N. G., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265-275 (1951).
- ¹⁴ Malkinson, A. M., and Ashworth, J. M., *Biochem. J.*, **134**, 311-319 (1973).

Carbohydrate binding proteins involved in phagocytosis by *Acanthamoeba*

THE soil sarcodinid *Acanthamoeba castellanii* grows readily in axenic culture^{1,2} or in monoxenic conditions with a variety of bacteria as food organisms³. The reduced growth of this organism in the absence of particulate material⁴ suggests that even under axenic conditions the formation of phagocytic vesicles is important in the uptake of nutrients, as in *Tetrahymena*⁵. We present evidence that the preferential uptake by *A. castellanii* of horse red blood cells over those from other sources⁶ is caused by binding of these cells to carbohydrate sensitive sites on the surface of the amoebae, and that these sites may function in the same way in the amoeba's natural habitat.

When horse erythrocytes were mixed with logarithmic phase cultures of *A. castellanii*, massive mixed-cell agglutination resulted. Such a reaction was absent in mixtures of amoebae with human (all ABO groups) or sheep red cells. A range of carbohydrates were tested for their ability to inhibit this agglutination (Table 1). The sensitivity of this reaction is similar to that of haemagglutination caused by bacterial type I fimbriae^{7,8}. Pretreatment of the horse red cells with concanavalin A (con A) (0.16 mg ml⁻¹) or with phytohaemagglutination (PHA) (Wellcome), did not abolish the mixed cell agglutination, although in the PHA-treated cells simple haemagglutination occurred.

The effect of mannose on the preferential uptake of horse red blood cells was investigated by incubating horse and sheep red blood cells with amoebae in a glucose medium and horse red blood cells in a similar medium containing mannose (Fig. 1). Clearly the inhibition of the agglutination reaction also inhibits the preferential uptake of horse cells.

Carbohydrate-binding proteins have been implicated in many biological activities. Rosen *et al.*^{9,10} have described such proteins on the surface of slime-mould amoebae and have suggested that they are important in the aggregation of these amoebae during plasmodium formation.

We suggest, therefore, that *A. castellanii* possesses mannose-sensitive carbohydrate binding sites on its surface and that the attachment of particles to these sites facilitates their phagocytosis. In contrast Allen *et al.*¹¹ have reported that exogenous lectin (con A) will bind bacteria to the surface of mouse macrophages but this does not lead to phagocytosis; indeed, bound lectin will inhibit the attachment of opsonised bacteria. This is not the case in *A. castellanii*, in which lectins increase phago-

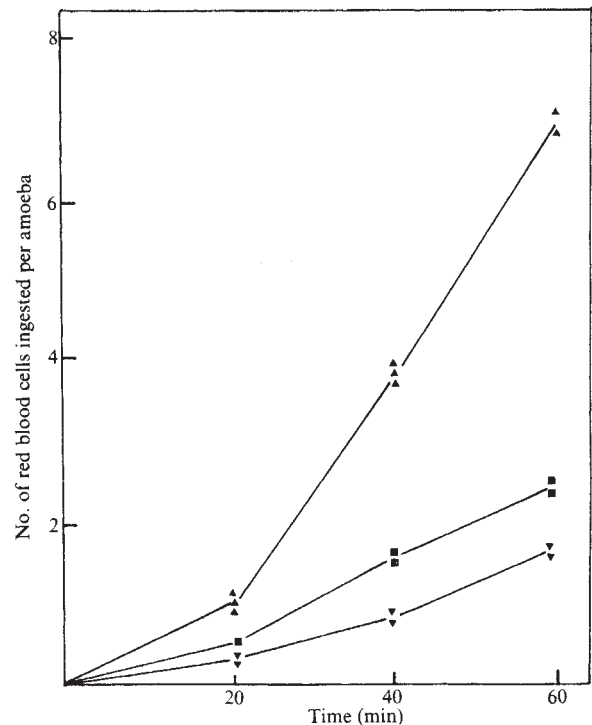


Fig. 1 Phagocytosis of horse and sheep red blood cells by *A. castellanii*. Amoebae from a log phase culture were washed in 0.9% (w/v) NaCl and 1 ml of a suspension put in each of six flasks. Medium (7 ml) containing proteose peptone 0.75% (w/v) and yeast extract 0.75% (w/v) were added to each flask, and glucose or mannose was added to a final concentration of 1.5% (w/v). Uptake of horse cells in glucose (▲) and in mannose (▼), is compared with uptake of sheep cells in glucose (■). Uptake was measured spectrophotometrically following hypotonic lysis of uningested red cells and acetic acid extraction of the haemoglobin from the amoebae as described⁶.

cytosis¹². The implication of the binding sites which we describe here to the ecology of the amoeba has been investigated by testing a variety of potential food organisms for their ability to form mixed cell aggregates with *A. castellanii* and to support monoxenic growth (Table 2). The ability of these particles to attach to the surface of the amoebae, thereby forming aggregates, correlates well with the occurrence of mannose in the

Table 1 Inhibition of mixed cell agglutination of *A. castellanii* and horse red blood cells by various carbohydrates

Carbohydrate	Minimum concentration inhibiting mixed cell agglutination
α-Methyl-D-mannoside	3 mM
D-mannose	3 mM
D-fructose	3 mM
Mannan (yeast)*	0.2%
D-glucose	not at 100 mM
D-galactose	not at 100 mM
D-arabinose	not at 100 mM
n-Acetyl glucosamine	not at 100 mM
Sucrose	not at 100 mM
Trehalose	not at 100 mM
L-sorbose	not at 100 mM
Melebiose	not at 100 mM
Inositol	not at 100 mM

Series of double dilutions of the carbohydrates were prepared in a salts solution containing (g l⁻¹) NaCl, 4.0; KCl, 0.2; MgSO₄, 0.05; CaCl₂, 0.07; NaHCO₃, 0.018 and 50 mM phosphate buffer, pH 7.2. A suspension (0.1 ml) of *A. castellanii* (10⁸ ml⁻¹) and 0.1 ml of a horse red blood cell suspension (10% v:v) were added to each well on a depression plate containing 0.4 ml of the carbohydrate solution. The plates were incubated at 30 °C and agglutination results read 20 min later. All carbohydrates were obtained from Sigma.

*Mannan from *Candida albicans* (a gift from E. G. V. Evans) agglutinated *A. castellanii* in the absence of red blood cells.

Table 2 Mixed cell agglutination of *A. castellanii* and monoxenic growth.

Organism particle	Mixed agglutination with <i>A. castellanii</i>	Supports monoxenic growth
Horse erythrocytes	++++	—
Sheep erythrocytes	—	—
Human erythrocytes	—	—
<i>Saccharomyces cerevisiae</i> CMD 53	+ + + +	+
<i>Saccharomyces carlsbergensis</i> IM1 80178	+++	+
<i>Candida utilis</i> CMD 39	++	+
<i>Shizosaccharomyces pombe</i>	+	+
<i>Pseudomonas aeruginosa</i> PAO 1	—	—
<i>Aerobacter aerogenes</i> NC1B 418	±	—
<i>Bacillus subtilis</i> NC1B 8057	—	+
<i>Staphylococcus aureus</i> CMD 99*	—	±
Polystyrene spheres 9 µm diameter	—	—

Agglutination was tested as described in Table 1. All the organisms were spread on to non-nutrient agar plates and allowed to dry; the plates were inoculated at their centres with drops of log phase culture of *A. castellanii*, incubated at 30 °C and examined daily. All the agglutination reactions described were sensitive to D-mannose, α-methyl-mannoside and fructose.

cell walls of the four yeast species tested. Although, under the conditions of our tests, organisms not so bound will support growth of the amoebae, they are present in greater numbers than would be food organisms in the natural environment of *Acanthamoeba*. At the lower temperatures and cell populations normally found in the soil, we suggest that phagocytosis would be greatly facilitated by preliminary binding of food organisms to the amoeba. It seems likely therefore that yeasts or mannan-containing fungi would form an important source of food for this soil amoeba.

Some strains of *Acanthamoeba* will cause meningoencephalitis in experimental animals¹³ and humans¹⁴⁻¹⁶. Stevens and Kaufman¹⁷ have already compared binding sites for con A in virulent and non-virulent strains. It would therefore be interesting to compare such strains with respect to the lectin-like activity which we describe here.

We thank Dr A. J. Griffiths and Mr I. J. C. Macintosh for discussions, and help in preparing the manuscript. R.C.B. is in receipt of a grant from the MRC as a postdoctoral research associate.

R. C. BROWN
H. BASS
J. P. COOMBS

Department of Microbiology,
University College,
Newport Road, Cardiff, UK

Received January 27, 1975.

- Adam, K. M. G., *J. gen. Microbiol.*, **21**, 519-529 (1959).
- Band, R. N., *J. gen. Microbiol.*, **21**, 80-95 (1959).
- Stout, J. D., and Heal, O. W., in *Soil biology* (edit. by Burgess, A., and Raw, F.), 179-181 (Academic, London and New York, 1967).
- Holst-Sorenson, H., and Rasmussen, L., *C. r. Trav. Lab., Carlsberg.*, **38**, 163-170 (1971).
- Rasmussen, L., *Nature*, **250**, 157-158 (1974).
- Rabinovitch, M., and De Stefano, M. J., *Expl Cell Res.*, **64**, 275-284 (1971).
- Duguid, J. P., and Gillies, R. R., *J. Path. Bact.*, **74**, 397-411 (1957).
- Old, D. C., *J. gen. Microbiol.*, **71**, 149-157 (1972).
- Rosen, S. D., Kafka, J. A., Simpson, D. L., and Barondes, S. H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2554 (1974).
- Rosen, S. D., Simpson, D. L., Rose, J. C., and Barondes, S. H., *Nature*, **252**, 128-130 (1974).
- Allen, J. M., Cook, G. M. W., and Poole, A. R., *Expl Cell Res.*, **68**, 466-471 (1971).
- Rabinovitch, M., and De Stefano, M. J., *Nature*, **234**, 414-415 (1971).
- Culbertson, C. G., *A. Rev. Microbiol.*, **25**, 231-254 (1971).
- Kenney, M., *Health Lab. Sci.*, **8**, 5-10 (1971).
- Robert, V. B., and Rorke, L. B., *Ann. intern. Med.*, **79**, 174-179 (1973).
- Jager, B. V., and Stamm, R. R., *Lancet*, **ii**, 1343-1345 (1972).
- Stevens, A. R., and Kaufman, A. E., *Nature*, **252**, 43-45 (1974).

Visual adaptation in butterflies

THE brilliant glow appearing in the compound eye of butterflies has intrigued investigators of insect eyes since the observations of Exner¹. Miller and Bernard have presented a convincing optical interpretation of the butterfly glow, that the tracheole basal to the rhabdom is modified in such a way as to

act as a quarter wavelength interference reflection filter (see refs 2-4). On light adaptation, the eye glow vanishes rapidly¹. Miller and Bernard have conjectured that this is caused by a distal migration of pigment granules in the pigment cells surrounding the crystalline cone².

Both the basal reflection theory and the pigment migration hypothesis have been challenged by Swihart *et al.*⁵ (highlighted recently⁶). They concluded that the phenomena can be attributed to reflections from corneal processes and photomechanical changes in the retinula cells^{5,7}, respectively. Comparison of our studies on the pupil mechanism of flies⁸⁻¹⁰ with similar investigations on the visual adaptation of butterflies¹¹ suggests that most of the data of Swihart *et al.*⁵ strongly support the earlier theories, if these are modified in one important respect—that the distal migration occurs not in the pigment cells but within the retinula cells themselves.

This view is presented diagrammatically in Fig. 1, showing an ommatidium of a butterfly. Since the rhabdom functions as an

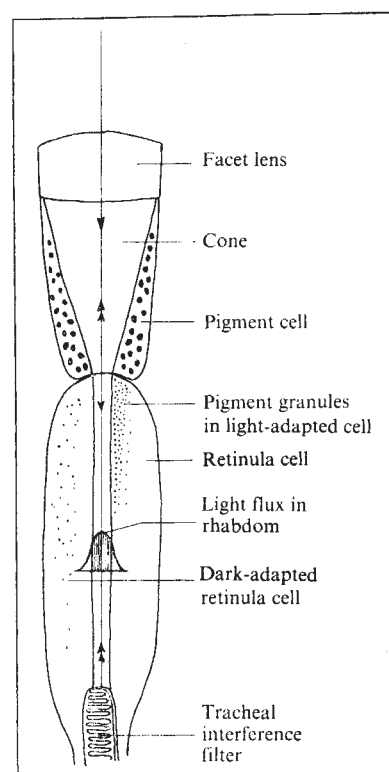


Fig. 1 An ommatidium of the compound eye of a butterfly. The dioptrical apparatus consists of a transparent facet lens and a cone which are surrounded by heavily pigmented "screening" pigment cells. The photoreceptor cells feature a specialised membrane structure, the rhabdomere, which contains the visual molecules. Within each ommatidium the rhabdomeres join in a fused rhabdom, an entity which functions as an optical wave guide¹¹⁻¹³. Proximal to the rhabdom an invaginated structure of the tracheal system creates a reflection interference filter. So light which enters the ommatidium through facet lens and cone after being propagated by the rhabdom is partially reflected by the basal reflection filter and so can by the same route leave the eye again. Obviously a fraction of the light propagated in the rhabdom is absorbed by the visual molecules but owing to the waveguide properties of the rhabdom light is also propagated outside the rhabdom boundary as indicated by the bell-shaped curve, representing schematically one of the possible light distributions. Hence pigment granules, existing in the photoreceptor cells as follows from electronmicroscopy (J. Tinbergen, unpublished), if localised near the boundary of the rhabdom, can also diminish the light flux, that is, by absorption as well as by scattering. By variation of the amount of pigment granules near the rhabdomere the light flux in the butterfly rhabdom can be regulated in an identical way to that occurring in other insects⁸⁻¹⁶. In the dark-adapted state (Fig. 1, left) the pigment granules are dispersed through the visual cell soma. On light adaptation (Fig. 1, right) the pigment granules will move towards the rhabdom until an equilibrium situation is reached (see refs 8, 12, 15, 16 and 22).

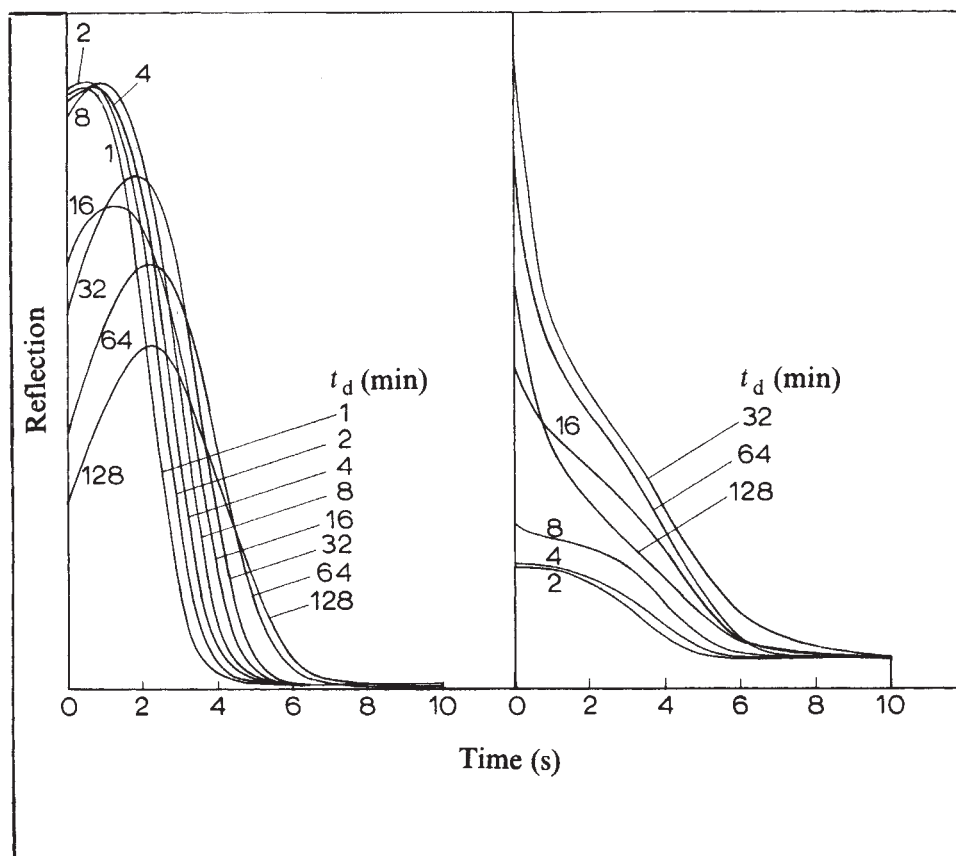


Fig. 2 Reflection of the compound eye of the small Tortoiseshell (*Aglaia urticae*, Nymphalidae). The curves represent the responses to 10 second pulses of intense light subsequent to a variable dark time t_d , which in turn followed just such a 10 second pulse. Irradiation of 584 nm (Fig. 2a) and 488 nm (Fig. 2b) was applied. The initial changes are caused by photochemical events, followed by reflection decreases which are probably due to the migration of absorbing pigment granules towards and against the butterfly rhabdom, thus fulfilling a pupil action. The pupil opens during the dark within 0.5–1 min. So the initial value of the reflection indicates the state of the visual pigment. It proves (Fig. 2a) that after prolonged dark adaptation the initial reflection value falls, pointing to dark regeneration of a green rhodopsin. This regeneration occurs with a biphasic process, since in the blue the initial reflection first increases but later on decreases again when the dark adaptation time rises above about half an hour (Fig. 2b). Possibly a violet absorbing intermediate is involved in the dark regeneration process.

optical waveguide the light flux reaching the visual molecules can be controlled by the pigment granules in the photoreceptor cells through regulation of their number near the boundary of the rhabdom (see refs 8–14); a pupil function is in this way fulfilled. As a result of the interference reflection filter created by the tracheole basal to the rhabdom, a fraction of the incident light will leave the eye again after a double passage through the rhabdom. This fraction of light is the glow visible in the butterfly eye. An implication of the outlined conception is that reflection measurements provide knowledge concerning both the visual pigment processes and the pigment migration inside the visual sense cells.

The butterfly glow and its disappearance are best seen in the centre of the deep-pseudopupil^{11–14}, using a vertical-illumination microscope. The observations (ref. 14, Fig. 18) convincingly support the view that the glow is in fact light leaving the rhabdom; and these results disagree completely with the recent proposal⁵ that the glow represents reflections arising from the ring of corneal processes surrounding the cone^{5,7}.

The experimental reflection curves shown in Fig. 2 were obtained from the small Tortoiseshell butterfly, *Aglaia urticae* (Nymphalidae). A saturating illumination (10 s) of wavelength λ was given after a variable dark-adaptation time t_d , which in turn followed a similar 10 s pulse. Figure 2a and b represent the superimposed recordings of the reflected light during light adaptation at $\lambda=584$ nm and $\lambda=488$ nm, respectively. An initial reflection increase (a) or an initial decrease (b), which becomes more prominent as t_d is increased, is followed in both cases by a considerable reflection decrease, the time course of which becomes longer with longer t_d .

These observations parallel transmission measurements from blowfly photoreceptors¹⁵. In the latter, intense illumination causes initial transmission changes resulting from photochemical processes in the visual pigment, and subsequently

there is a decrease in transmission which is the consequence of absorbing and scattering pigment granules accumulating near the rhabdomere acting as a light guide^{8–10}. In the fly, the rate of fall in transmission also slows down with prolonged dark time t_d .

Mutatis mutandis, it is reasonable to assign the initial reflection changes in Fig. 2 to photochemical processes and to interpret the subsequent fall in reflection as induced by retinula cell pigment granula gathering near the boundary of the butterfly rhabdom, in agreement with the hypothesis stated above. Swihart *et al.*⁵ remark that "should the basal theory be correct, one would imagine that the basal reflections would increase in apparent intensity as the visual pigments were bleached"⁵.

Yet, bleaching, implying photolysis of visual pigment, cannot be the correct explanation for the initial events of both Fig. 2a and b. Rather the transients can be interpreted as representing photochemical conversions of a green-absorbing rhodopsin P525 into a blue-absorbing metarhodopsin M480 and vice versa as has been concluded for the related moths^{17,18}; similar visual pigment processes have been reported for other invertebrates^{19,20}.

A photochemical equilibrium between the two pigment states will be established at light adaptation. This equilibrium, however, is obviously not maintained during the dark. The initial value of the reflection at 584 nm continuously decreases with increasing dark time t_d which implies an increase in absorption at that wavelength, or a regeneration in the dark of the green rhodopsin. Although this is not apparent at 584 nm (Fig. 2a) the dark process must involve two stages, since at 488 nm (Fig. 2b) the initial value of the reflection increases with t_d only during the first half hour of darkness and decreases again after a longer period in the dark.

The biphasic dark process can be explained by several

alternatives, such as : the blue absorbing metarhodopsin being a quasi-thermostable photoproduct being converted by way of a violet-absorbing intermediate into the native rhodopsin; a blue and a violet metarhodopsin could coexist, each state having its own dark conversion rate; dark conversions of other rhodopsins than that absorbing green could be involved. The decision as to which alternative must be ruled out requires a detailed analysis, but the unequivocal establishment of the dark-conversions of the butterfly visual pigment(s) would provide a valuable example of the very scantily known *in vivo* dark processes of invertebrate visual pigments²¹.

The assumed existence of a green rhodopsin in butterfly eyes is in good agreement with Swihart *et al.*⁵ that the action spectrum for the extinction of the butterfly glow points to a visual pigment absorbing most strongly at 520–540 nm. Hence, the pupil of butterflies could be driven by a force coupled to the receptor potential in the retinula cells in a manner corresponding to the pupil mechanism of flies^{8,12,15,16,22}.

A further parallel between fly and butterfly pupils may be put forward. Illumination of fly photoreceptor cells with intense blue light predominantly converts fly rhodopsin P495 into metarhodopsin M580, resulting in a delayed dark adaptation, which is counteracted by the opposite photopigment conversion^{22,23}. Furthermore, the pupil of flies absorbs most strongly at just those blue wavelengths which most effectively delay dark adaptation^{9,22}. We therefore conclude that the fly's pupil not only controls the light flux in the photoreceptor but also its spectral distribution, so as to improve the speed of adaptation.

Antagonistic effects of visual pigment conversions on the adaptation of photoreceptor cells have been reported also for the barnacle²⁰, having a green rhodopsin P532 which is photo-interconvertible with a metarhodopsin M495. In the barnacle it is red light, favouring conversion of rhodopsin to metarhodopsin, which seems to be most effective in delaying dark adaptation of the visual sense cells. In the butterfly delayed dark adaptation can also be induced with intense red illumination (D.G.S., unpublished). Hence, from our knowledge of the pupil of flies we might expect that the butterfly's pupil absorbs extremely in the red to improve the speed of dark adaptation in those photoreceptor cells possessing a green rhodopsin.

The absorbance spectrum of the pupil of the small Tortoiseshell butterfly (Fig. 3) shows little absorbance in the blue and extreme absorbance in the red, confirming the stated prediction. Since we have observed in other butterflies pupil spectra as well as photochemical processes very similar to those reported above, we conclude that, although the visual pigments and the pupil of butterflies and flies seem to have quite different spectral properties, in at least these insect species the pupil may have an

essential function in the speeding up of the dark adaptation after intense illumination. It also seems that the butterfly experiments can be satisfactorily interpreted by a combination of the basal reflection theory with the hypothesis of the intracellular pupil.

The collaboration of Professor J. W. Kuiper and of J. H. Flokstra, J. Tinbergen and A. Zantema is acknowledged.

D. G. STAVENGA

Biophysical Department,
Laboratorium voor Algemene Natuurkunde,
Rijksuniversiteit Groningen,
Groningen, The Netherlands

Received October 15, 1974; revised January 13, 1975.

- ¹ Exner, S., *Die Physiologie der facettierten Augen von Krebsen und Insekten* (Franz. Deuticke, Leipzig-Wien, 1891).
- ² Miller, W. H., and Bernard, G. D., *J. Ultrastruct. Res.*, **24**, 286–294 (1968).
- ³ Miller, W. H., Bernard, G. D., and Allen, J. L., *Science*, **162**, 760–767 (1968).
- ⁴ Land, M. F., *Prog. Biophys.*, **24**, 75–106 (1972).
- ⁵ Swihart, S. L., Gordon, W. C., and Machwart, R. J., *J. Insect Physiol.*, **20**, 359–381 (1974).
- ⁶ *Nature*, **250**, 188 (1974).
- ⁷ Swihart, S. L., *J. Insect Physiol.*, **20**, 1027–1036 (1974).
- ⁸ Stavenga, D. G., *Proc. Int. Union Physiol. Sc.*, **IX**, 532, 25th Int. Congr. Munich (1971).
- ⁹ Stavenga, D. G., Zantema, A., and Kuiper, J. W., in *Biochemistry and Physiology of Visual Pigments* (edit. by Langer, H.) 175–180 (Springer, Berlin, 1973).
- ¹⁰ Stavenga, D. G., *J. comp. Physiol.*, **91**, 417–426 (1974).
- ¹¹ Stavenga, D. G., *Vision Res.*, **15**, 323–330 (1975).
- ¹² Kirschfeld, K., and Franceschini, N., *Kybernetik*, **6**, 13–22 (1969).
- ¹³ Snyder, A. W., and Horridge, G. A., *J. comp. Physiol.*, **81**, 1–8 (1972).
- ¹⁴ Franceschini, N., and Kirschfeld, K., *Kybernetik*, **9**, 159–182 (1971).
- ¹⁵ Stavenga, D. G., in *Receptor Optics* (edit. by Snyder, A. W., and Menzel, R.), (Springer, Berlin, in the press).
- ¹⁶ Franceschini, N., in *Information Processing in Visual Systems of Arthropods* (edit. by Wehner, R.), 75–82 (Springer, Berlin, 1972).
- ¹⁷ Hamdorf, K., Paulsen, R., and Schwemer, J., in *Biochemistry and Physiology of Visual Pigments* (edit. by Langer, H.), 155–166 (Springer, Berlin, 1973).
- ¹⁸ Hamdorf, K., Höglund, G., and Langer, H., *J. comp. Physiol.*, **86**, 247–263 (1973).
- ¹⁹ Brown, P. K., and White, R. H., *J. gen. Physiol.*, **59**, 401–414 (1972).
- ²⁰ Minke, B., Hochstein, S., and Hillman, P., *J. gen. Physiol.*, **62**, 105–128 (1973).
- ²¹ Goldsmith, T. H., and Bernard, G. D., in *Physiology of Insecta*, **II**, 165–272 (Academic, San Francisco, 1974).
- ²² Stavenga, D. G., Flokstra, J. H., and Kuiper, J. W., *Nature*, **253**, 740–742 (1975).
- ²³ Muijsers, H., Leutscher-Hazelhoff, J. T., Stavenga, D. G., and Kuiper, J. W., *Nature* (in the press).

Evidence for hormonal control of integumentary water loss in cockroaches

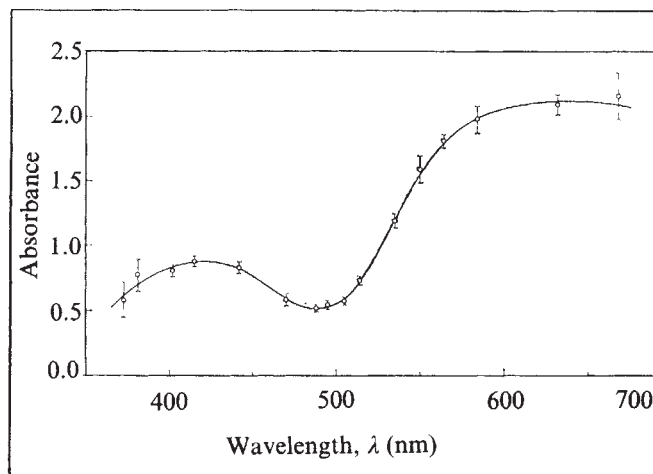
THE susceptibility of terrestrial insects to desiccation is minimised by extremely effective integumentary waterproofing, which results from the presence of remarkably impermeable epicuticular lipids¹ and, it is suggested, from the properties of the underlying epidermal cells². On the basis of rather limited information^{3,4} it has, in addition, been suggested that the rate of integumentary water loss may be regulated, the degree of permeability depending on the ambient humidity².

We now have evidence that integumentary waterproofing may be hormonally controlled. Weight loss is more rapid in decapitated cockroaches than in normal ones or in those in which nervous connection with the brain has been severed (Fig. 1). The involvement of a blood-borne factor is also indicated by the significant reduction in apparent water loss after injection of brain extract (Fig. 2), or to a lesser extent corpus cardiacum extract, into decapitated individuals.

That the accelerated water loss induced by decapitation occurs largely through the integument, and not through the excretory or respiratory systems, is deduced from the following evidence. First, anal blocking did not reduce the rate of waterloss in decapitated individuals. Second, the activity of thoracic spiracles was reduced following decapitation. Third, exposure to 5% CO₂ greatly increased spiracular opening, in both normal and decapitated cockroaches, but did not abolish the more rapid water loss in the latter. Finally, spiracular blocking did not significantly reduce water loss in decapitated individuals.

The accelerated integumentary water loss induced by

Fig. 3 Absorbance spectrum of the pupil of the small Tortoiseshell *Aglais urticae*. The graph represents the difference in absorbance between the 1-min dark-adapted state and the saturating light-adapted state.



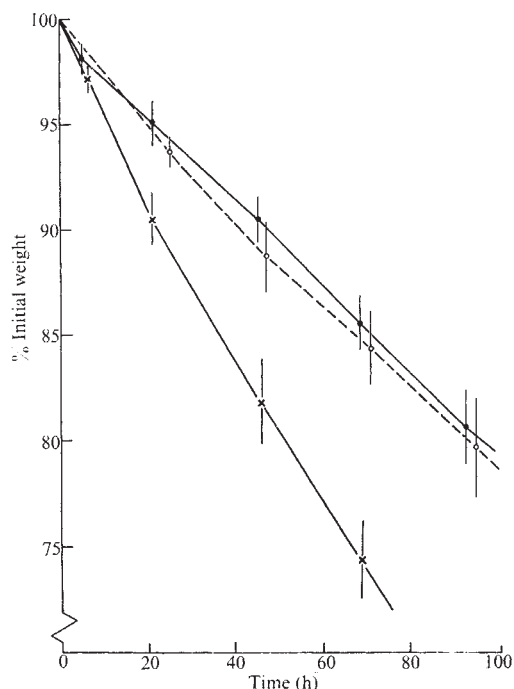


Fig. 1 Effects of decapitation and severance of the neck connective on the decline in weight of individuals maintained at 54–55% r.h. In this, and subsequent diagrams, the symbols indicate the mean of measurements made on ten individuals, the vertical lines indicating the extent of twice the standard error of the mean. ●, Normal insects; ○, neck connective severed; ×, headless insects.

decapitation could have resulted from nonspecific effects on the permeability of the epidermis, perhaps through an increase of intercellular diffusion resulting from changes in the lateral junctional complexes of epidermal cells (see ref. 2). The cation permeability of the cellular layer of the nerve sheath, however, was unaffected by decapitation: the extraneuronal potentials, and absence of effects on

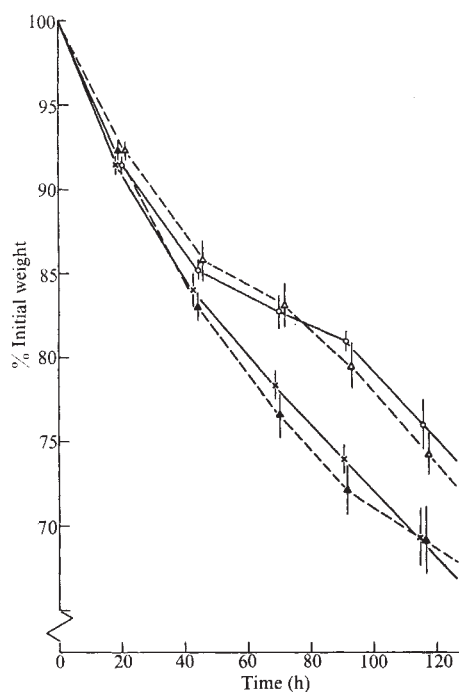


Fig. 2 The effects of injection of brain extract (1 brain + 50 µl saline) on the decline in weight of decapitated insects compared with that of normal and control individuals maintained at 48–50% r.h. ○, Normal insects; △, headless + brain-extract; ▲, headless + Ringer solution; × headless.

the action potentials induced by high-potassium saline were similar to those of normal individuals⁵.

It is also possible that the apparent blood-borne factor might act by changing the synthesis and deposition of epicuticular lipids. The incorporation of ¹⁴C-acetate into epicuticular lipids⁶ following injection into the haemolymph, however, was not significantly reduced in decapitated individuals. The radioactivity of the lipids washed from the body surface of normal individuals with Triton X and toluene solvent 24 h after injection of 0.5 µCi into the haemolymph was $1,539 \pm 156$ ($n=6$) d.p.m. compared with $1,436 \pm 260$ ($n=5$) d.p.m. for decapitated ones.

It is, thus, difficult to avoid the conclusion that integumentary permeability may be under neuroendocrine control. The apparent involvement of both the brain and the corpus cardiacum suggests a similarity with other systems in which neurosecretory material is synthesised within neuronal cell bodies in the brain and is then transported along specialised axons to the corpus cardiacum (see ref. 7).

The prolonged effect of injected brain extract (Fig. 2) in reducing water loss suggests that the blood-borne factor is not rapidly metabolised in decapitated individuals. A rapid inactivation must, however, be assumed in normal individuals, for injection of brain extract 24 h before the application of a neck ligature produced no appreciable reduction in water loss (Fig. 3). This situation seems to be analogous

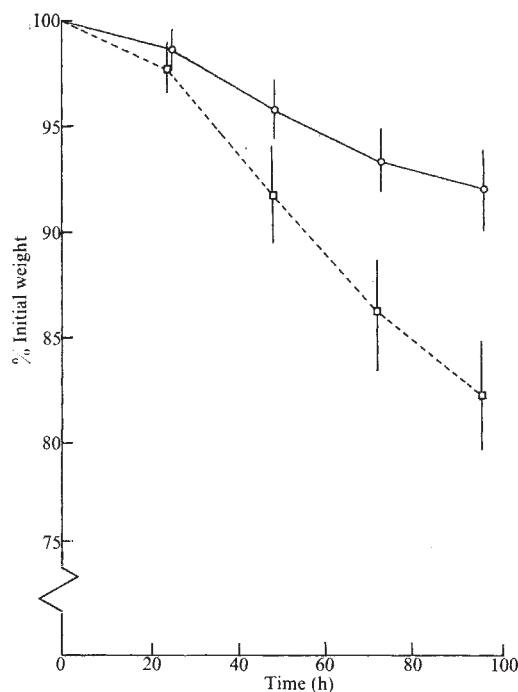


Fig. 3 The effect of the application of a neck ligature 24 h after the injection of brain extract (1 brain + 50 µl saline) on the decline in weight at 48–50% r.h. ○, Normal insects; □, brain extract injected 24 h before application of neck ligature.

to that for the diuretic hormone in the tsetse fly, in which it is proposed that an inactivating factor is released into the haemolymph from the thoracic ganglion together with the hormone⁸. The persistence of the effects of brain extract in decapitated insects could either mean that the inactivating factor is unstable following extraction, or that it is normally synthesised in the brain only in response to high levels of the waterproofing factor in the haemolymph.

Removal of the frontal ganglion or severance of the frontal connectives increases the rate of water loss from cockroaches and it is suggested that the frontal ganglion receives an input from osmoreceptors⁹. The frontal ganglion could also be involved in mediating the release of water-

proofing factor from the brain by way of the corpus cardiacum.

Little is known concerning the mode of action of the postulated blood-borne factor. It has been shown, however, that decapitation has little effect on the electrical resistance of the integument¹⁰. The resistance for normal pronotal integument averaged $3,571 \pm 1,324 \Omega \text{ cm}^2$ as compared with $3,661 \pm 1,020 \Omega \text{ cm}^2$ measured in decapitated individuals. The observations would accord with the idea, for example, that transpiration is increased by alterations in an oriented epicuticular lipid layer¹ which may be insufficient to affect permeability to inorganic cations and thus the flow of current across the integument.

The physiological role of a neuroendocrine control of integumentary transpiration remains to be elucidated. It would be an obvious advantage for insects to be maximally waterproof in dry conditions. It is conceivable, however, to cite a single example, that under conditions of excessive water intake (such as might occur with a liquid or semi-liquid diet of low nitrogen content) it could be physiologically advantageous to increase the rate of integumentary transpiration. Alternatively, it could be that this apparent neuroendocrine control ensures that the organisation of the epicuticular lipids is maintained so as to ensure maximal waterproofing. An example of such a maintenance function could be the controlled release of the antioxidant, protocatechuic acid, which is necessary to prevent the degradation and polymerisation of epicuticular lipids¹¹.

J. E. TREHERNE

P. G. WILLMER

ARC Unit of Invertebrate Chemistry and Physiology,

Department of Zoology,

University of Cambridge, Cambridge, UK

Received January 7, 1975.

¹ Beament, J. W. L., in *Advances in Insect Physiology*, (edit. by Beament, J. W. L., Treherne, J. E., and Wigglesworth, V. B.), 2, 67–129 (Academic, London and New York, 1964).

² Berridge, M. J., in *Chemical Zoology* (edit. by Florkin, M., and Scheer, B. T.), 5, 287–319 (Academic, New York and London, 1970).

³ Loveridge, J. P., *J. exp. Biol.*, 49, 1–13 (1968).

⁴ Bursell, E., *J. exp. Biol.*, 32, 238–255 (1955).

⁵ Pichon, Y., and Treherne, J. E., *J. exp. Biol.*, 53, 485–493 (1970).

⁶ Diehl, P. A., *Nature*, 243, 469–470 (1973).

⁷ Maddrell, S. H. P., in *Insect Neurobiology* (edit. by Treherne, J. E.), 307–357 (North Holland, Amsterdam, 1974).

⁸ Gee, J. D., thesis, Univ. Cambridge (1974).

⁹ Penzlin, H., and Stölzner, W., *Experientia*, 27, 390–391 (1971).

¹⁰ Schele, P. O., in *Experiments in Physiology and Biochemistry*, (edit. by Kerkut, G.), 3, 181–210 (Academic, London and New York, 1970).

¹¹ Atkinson, P. W., and Gilbey, A. R., *Science*, 168, 442 (1970).



Fig. 1 An example of bilateral cannula tracks with the tips in the sulcal prefrontal cortex. For the cannula implantation, level-head stereotaxic coordinates⁶ were used. Adult male, hooded rats (300 g) were cannulated and injected as described elsewhere⁴. Each rat was implanted bilaterally with stainless steel guide cannulae, fitted with an obturator. The latter was removed and replaced with an injection cannula before an injection experiment. Ten rats were implanted bilaterally with cannulae in the sulcal prefrontal cortex. The stereotaxic coordinates of each cannula tip were 2.3 mm anterior to the bregma, 3.5 mm lateral to the sagittal sinus, and 4.0 mm beneath the dorsal surface of the brain (+2.3; 3.5; 4.0 mm down). Either 5% procaine hydrochloride in 0.9% saline or saline alone was injected bilaterally. Responses to aversive footshock were determined using a 'flinch-jump' test⁵. Each rat received six series of ten shocks per series, in which scrambled electrical shocks (0.5 s duration) were delivered at 15 s intervals to the floor of the test box. The shock intensities in each series were ten consecutive values from the range 0.13 to 1.60 mA, with 60 s between each series. Shocks were delivered in ascending and descending order on alternate series. The rat's response to each shock was recorded as either no response, flinch (startle, including forepaw-shaking), or jump (both rear paws leave floor). The jump response alone seems to be related to specifically painful stimulation, since morphine greatly increases the jump threshold without affecting the flinch threshold⁵. The flinch and jump thresholds were noted for each series, and the mean values over the six series calculated. The rat was then removed, injected bilaterally with 1.0 μl 5% procaine hydrochloride, and immediately returned to the test box. Six more shock series were run to determine postinjection flinch and jump thresholds. After 30 min, the effects of the procaine having dissipated, the rat was re-injected with 2.0 μl 5% procaine hydrochloride, and a second series of six postinjection series were run. The same procedure was repeated no sooner than 48 h afterwards, to determine the effects of the injection of 0.9% saline. For statistical analysis, the threshold results obtained for the pre- and postinjection series were compared using a correlated *t* test (two-tailed).

Anaesthetisation of prefrontal cortex and response to noxious stimulation

REPORTS indicate that responses to pain may be modified by damage to the frontal lobes (see refs 1 and 2). Nevertheless, neither the nature of the change nor the degree to which any effect of damage is localised anatomically has been firmly established. I have systematically examined the function of the prefrontal cortex in response to an aversive stimulus in the rat. Small quantities of a short-acting anaesthetic, procaine hydrochloride, were injected to block directly the neural function of specific areas within the prefrontal cortex. The prefrontal cortex in the rat occupies both a lateral region, the dorsal bank of the rhinal sulcus, and a medial region, the pregenual medial wall of the hemisphere³. In the rat, the prefrontal cortex is involved in brain-stimulation reward⁴. I suggest that, in addition, the prefrontal cortex acts to limit the response to painful stimulation, and that this anti-nociceptive function is localised in or near the sulcal region.

Following the bilateral injection of 1.0 μl 5% procaine hydrochloride (see legend to Fig. 1), the preinjection jump threshold of 0.86 ± 0.22 mA (mean $\pm$ s.d.) was reduced to 0.77 ± 0.19 mA ($t=6.2$, $P<0.001$), and after bilateral injection of 2 μl , it was further reduced to 0.73 ± 0.21 mA ($t=6.9$, $P<0.001$). Every animal exhibited a reduction in jump threshold following both the 1.0 and 2.0 μl bilateral injections. The

flinch threshold, in contrast, remained unchanged. The pre-injection flinch threshold was 0.19 ± 0.05 mA, after bilateral injection of 1.0 μl procaine hydrochloride 0.18 ± 0.03 mA, and after 2.0 μl bilaterally 0.18 ± 0.03 mA (non-significant differences). In the saline control experiments, the preinjection jump threshold of 0.86 ± 0.22 mA did not differ significantly from the threshold value 0.87 ± 0.22 mA after the injection of 2.0 μl saline bilaterally. The lowered jump threshold which followed bilateral anaesthetisation of the sulcal prefrontal cortex did not result from a nonspecific reduction in response thresholds, since first, the flinch thresholds remained unchanged, and secondly, such anaesthetisation has been shown to increase self-stimulation thresholds⁴. Thirdly, while the anaesthetic was effective, the general behaviour of the animals seemed to be unaffected (see also ref. 4). Bilateral procaine injections seemed to be necessary since, in subsequent work, no effect of unilateral procaine injection on the jump threshold was observed. An example of histology which shows that the tips of the cannulae were placed bilaterally in the sulcal region is shown in Fig. 1.

Additional experiments were run to determine the degree

of localisation of the effect of bilateral anaesthetisation on the jump response to footshock. Four rats were implanted with bilateral guide cannulae aimed at the medial prefrontal cortex ($+2.3 \pm 2.0$: 2.4 mm down, angled 28° towards the midline) and three rats with bilateral cannulae aimed 1.0 mm behind the effective sulcal placement. The animals were tested as above. Bilateral injections of 1.0–2.0 μ l procaine hydrochloride, or 0.9% saline, had no effect on either the jump or the flinch thresholds in both groups of animals. This series of experiments demonstrates conclusively that bilateral anaesthetisation of a region in or near the sulcal prefrontal cortex lowers the threshold of the jump response elicited by footshock. The jump response, in contrast to the flinch response, seems to be elicited by painful consequences of footshock⁶. A region specifically in or near the sulcal prefrontal cortex seems to be implicated, since procaine injected into either the medial prefrontal cortex or a site 1.0 mm behind the effective site had no effect on the jump threshold. The conclusion that, in the intact rat, a region in or near the sulcal prefrontal cortex functions to limit the response to painful footshock, is supported by the results of these experiments. Also of interest was whether the prefrontal cortex is a site of action of the potent analgesic drug, morphine. Since bilateral injections of morphine sulphate (4% solution) into either the sulcal or the medial prefrontal cortex do not affect the jump threshold (S.J.C., unpublished), it would seem unlikely that morphine analgesia⁵ involves any action within the prefrontal cortex.

I thank M. C. Ostermeyer, C. Henry and Mrs J. Curwell for assistance.

STEVEN J. COOPER

Department of Psychology,
The Queen's University of Belfast,
Belfast BT7 1NN, Northern Ireland

Received January 20, 1975.

- 1 Fulton, J. F., *Frontal Lobotomy and Affective Behaviour* (Chapman and Hall, London, 1951).
- 2 Melzack, R., *Puzzle of Pain* (Penguin, Harmondsworth, 1973).
- 3 Leonard, C. M., *Brain Res.*, **12**, 321–343 (1969).
- 4 Rolls, E. T., and Cooper, S. J., *Brain Res.*, **60**, 351–368 (1973); *Physiol. Behav.*, **12**, 563–571 (1974); *Expl Neurol.*, **42**, 687–699 (1974).
- 5 Evans, W. O., *Psychopharmacologia*, **2**, 318–325 (1961).
- 6 König, J. F. R., and Klippel, R. A., *Rat Brain* (Williams and Wilkins, Baltimore, 1963).

Pre- and postsynaptic components in effect of drugs with α adrenoceptor affinity

ACTIVATION of α adrenoceptors on noradrenergic nerve endings is thought to inhibit the release of noradrenaline by nerve impulses^{1,2}. The existence of a presynaptic receptor system introduces a new concept to the pharmacology of drugs with affinity for α adrenoceptors. Only when the presynaptic nerve does not secrete, as for example in the absence of impulses, may one assume *a priori* that the observed postsynaptic effects of these drugs result from their interaction with postsynaptic receptors. When action potentials arrive at the nerve endings, noradrenaline is released and keeps the postsynaptic receptors in a certain degree of excitation. Drugs which activate α adrenoceptors may enhance this excitation by their postsynaptic effects, or reduce it by their presynaptic actions. Conversely, α adreno-lytic drugs may diminish the excitation by postsynaptic blockade, or enhance it by facilitation of release. If the postsynaptic receptors are of the β type, the prevailing action is postsynaptic; thus, drugs which activate α adrenoceptors reduce^{3–5}, whereas, less regularly, α adreno-lytic drugs increase⁶ cardiac responses to sympathetic nerve stimulation. If the postsynaptic receptors are of the α type, the prevailing effect of α adreno-lytic drugs is postsynaptic blockade⁷; we report here that the effects of drugs which activate α adrenoceptors are diverse.

The experiments were performed on rabbit main pulmonary arteries, in which postsynaptic α adrenoceptors mediate

vasoconstriction. Artery strips were superfused with Krebs–Henseleit solution containing propranolol, cocaine and corticosterone to block β adrenoceptors and the neuronal and extraneuronal uptake of noradrenaline, respectively. Actions on postsynaptic α adrenoceptors were studied by determining dose–response curves in the absence of nerve impulses. For the separate study of presynaptic actions, arteries were preincubated with ^3H -noradrenaline, and drug effects on the overflow of tritium evoked by transmural electrical stimulation were measured. The mode of stimulation used selectively excites the adrenergic nerves of the arteries; the stimulation-induced overflow of tritium reflects the stimulation-induced secretion of noradrenaline from nerve terminals^{8,9}. To study the influence on postsynaptic cells during active neurotransmission, frequency–response curves were elicited before and after the addition of the drug.

Isolated pre- and postsynaptic actions of oxymetazoline and phenylephrine are shown in Fig. 1. Oxymetazoline preferentially activated presynaptic α adrenoceptors and thus inhibited release, whereas phenylephrine preferentially activated postsynaptic receptors and thus induced contraction. Methoxamine behaved like phenylephrine; clonidine has been shown to behave like oxymetazoline⁸. The differences in the relative potencies of these drugs may reflect structural differences in pre- and postsynaptic binding sites.

Stepwise increases in the frequency of transmural stimulation induced stepwise contraction, maximal at 16 Hz. Figure 2 illustrates the influence of drugs which activate α adrenoceptors at low concentrations which, in the absence of impulse flow, caused contractions ranging between 1.0% and 3.7% of the maximal response to noradrenaline. As would be predicted from their relative pre- and postsynaptic potencies, oxymetazoline and clonidine reduced, whereas phenylephrine and methoxamine enhanced neurogenic vasoconstriction. The effect of α adreno-ceptor-activating drugs in inhibiting noradrenaline release, declines as the frequency of stimulation is increased^{3,5}. Accordingly, clonidine and oxymetazoline inhibited smooth muscle responses to stimulation at low, but not at high frequency. The

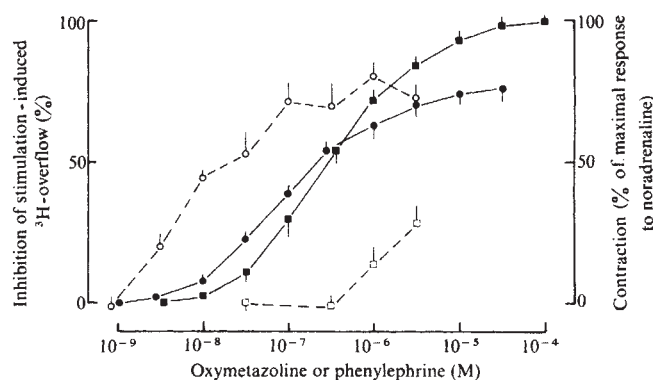


Fig. 1 Pre- and postsynaptic actions of oxymetazoline (○, ●) and phenylephrine (□, ■) in the rabbit pulmonary artery. Left ordinate, presynaptic action (○, □). Arteries were preincubated with 1.5×10^{-6} M (—) ^3H -noradrenaline for 1 h and then superfused with fresh medium containing 3×10^{-5} M cocaine, 4×10^{-5} M corticosterone and 4×10^{-6} M propranolol. Each strip was stimulated five times (S_1 – S_5) for 3 min with pulses of 0.3 ms duration and 150 mA current strength at 2 Hz. Stimulation periods started after 126, 144, 162, 180 and 198 min of superfusion. Oxymetazoline or phenylephrine were added 12 min before S_4 . Phenylephrine concentrations exceeding 3×10^{-6} M could not be tested, since they accelerated basal tritium outflow. In control experiments, the ratio between the overflow of tritium evoked by S_4 and that evoked by S_5 was 0.97 ± 0.03 ($n = 11$). The ordinate indicates percentage decreases of the ratio caused by the drugs. Each point is the mean $\pm$ s.e.m. of four to eight experiments. Right ordinate, postsynaptic action (●, ■). Two dose–response curves were determined on each strip, first a curve for noradrenaline, then a curve for oxymetazoline or phenylephrine. Results are corrected for the increase of contractions with time⁸. Values are the means $\pm$ s.e.m. of five dose–response curves for oxymetazoline and phenylephrine.

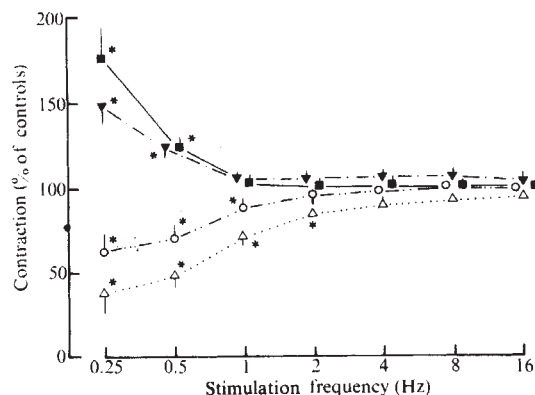


Fig. 2 Influence of α adrenoceptor-activating drugs on neurogenic contraction of the rabbit pulmonary artery. Arteries were superfused with medium containing 3×10^{-5} M cocaine, 4×10^{-5} M corticosterone and 4×10^{-6} M propranolol. Two frequency-response curves (FRC) were elicited on each strip (FRC₁, FRC₂). The following drugs were added 45 min before FRC₂: Δ , 3×10^{-8} M clonidine, $n = 7$; $\circ$, 3×10^{-9} M oxymetazoline, $n = 8$; ∇ , 10^{-7} M methoxamine, $n = 5$; $\blacksquare$, 10^{-8} M phenylephrine, $n = 10$. For each frequency, the ratio was calculated between the contraction during FRC₂ including drug-induced basal tension, and that during FRC₁. In control experiments, ratios were slightly less than (at 0.25 and 0.5 Hz) or greater than 1. Ratios obtained in the presence of drugs are expressed as a percentage of control ratios (ordinate). Means $\pm$ s.e.m. Significant differences from control: * $P < 0.05$.

inhibition was observed with 10^{-8} – 10^{-7} M clonidine⁸ and 10^{-9} – 3×10^{-9} M oxymetazoline; at higher concentrations, post-synaptic receptor activation prevailed and vasoconstriction was enhanced. In contrast, phenylephrine and methoxamine uniformly enhanced vasoconstriction in response to low frequency stimulation at concentrations from 3×10^{-9} – 3×10^{-6} M and 3×10^{-8} – 3×10^{-7} M, respectively.

In vivo, action potentials continuously arrive at nerve endings. Our results demonstrate that in this situation a presynaptic component significantly contributes to the overall postsynaptic effect of some drugs activating α adrenoceptors, even if postsynaptic receptors are of the α type. Selective pre- and postsynaptic receptor activation may also explain the diverse effects of α sympathomimetic drugs on the response of vas deferens to stimulation of its motor nerves^{5,10–12}. Detailed analyses of pre- and postsynaptic components may lead to a more complete understanding of the pharmacology of drugs that interact with α adrenoceptors.

We thank Miss B. Piel and Mr E. Hagelskamp for technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft.

KLAUS STARKE
TAKAHICO ENDO
HANS DETLEF TAUBE

Pharmakologisches Institut,
Klinikum der Universität Essen,
Hufelandstrasse 55,
D-4300 Essen,
Federal Republic of Germany

Received January 20, 1975.

- Langer, S. Z., *Biochem. Pharmacol.*, **23**, 1793–1800 (1974).
- Starke, K., in *Frontiers in Catecholamine Research* (edit by Usdin, E., and Synder, S. H.), 561–565 (Pergamon, Oxford, 1973).
- Starke, K., *Archs Pharmacol.*, **274**, 18–45 (1972).
- Armstrong, J. M., and Boura, A. L. A., *Br. J. Pharmacol.*, **47**, 850–852 (1973).
- Vizi, E. S., Somogyi, G. T., Hadházy, P., and Knoll, J., *Archs Pharmacol.*, **280**, 79–91 (1973).
- Farah, M. B., and Langer, S. Z., *Br. J. Pharmacol.*, **52**, 549–557 (1974).
- Dubocovich, M. L., and Langer, S. Z., *J. Physiol. Lond.*, **237**, 505–519 (1974).
- Starke, K., Montel, H., Gayk, W., and Merker, R., *Archs Pharmacol.*, **285**, 133–150 (1974).
- Su, C., and Bevan, J. A., *J. Pharmac. exp. Ther.*, **172**, 62–68 (1970).
- Ambache, N., and Zar, M. A., *J. Physiol. Lond.*, **216**, 359–389 (1971).
- Farnebo, L. O., and Malmfors, T., *Acta Physiol. scand.*, Suppl., **371**, 1–18 (1971).
- Sjöstrand, N. O., *Acta Physiol. scand.*, **89**, 10–18 (1973).

Affinity partitioning of acetylcholine receptor enriched membranes and their purification

AFFINITY chromatography, which uses a specific ligand coupled to a solid matrix to adsorb selectively a macromolecule, has been used extensively in the purification of soluble proteins¹. Although the purification of cells or cellular membrane fragments containing surface receptors has been attempted with this procedure^{2–15}, the approach has been less successful because of difficulties in eluting bound particulate substances from the solid matrix. Recently, we described a method called affinity partitioning¹⁶ for separating soluble proteins in aqueous polymer two-phase systems¹⁷ by adding a polymer-ligand with a relatively high affinity for a binding site on the protein to be purified, and a solubility preference for one of the phases. Since cells and cell fragments^{18,19} can be partitioned and recovered from aqueous polymer two-phase systems, it seemed possible that the principle of affinity partitioning could be used in their fractionation. With the absence of a solid matrix, problems of recovery would be obviated. We describe here the use of polymer coupled to a ligand that binds to the nicotinic cholinergic receptor site to purify cholinergic receptor enriched membranes derived from electroplax of *Torpedo californica*.

This membrane system was used to evaluate application of affinity partitioning to particulates because of the following favourable characteristics: first, the membranes contain unusually high receptor densities (up to $30,000 \mu\text{m}^{-1}$)^{20,21}; second, there is detailed knowledge of the ligand specificity and molecular properties of the binding site²²; third, differential and sucrose density gradient centrifugation have shown²³ that discrete membrane fragments enriched in nicotinic cholinergic

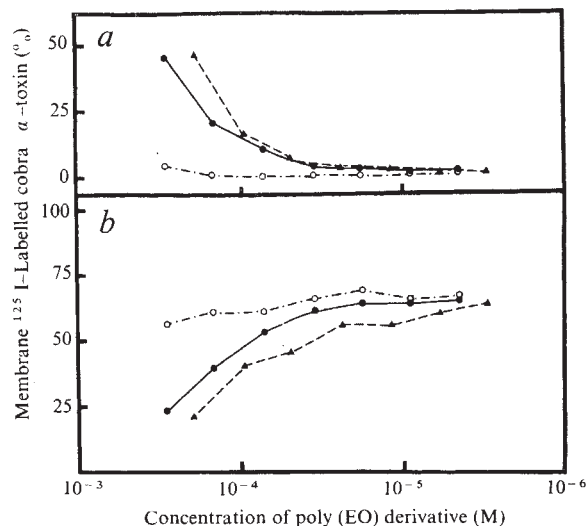


Fig. 1 Distribution of membrane-bound ^{125}I -labelled α -toxin in presence of various concentrations of MA-poly(EO) ($\circ$), TMA-poly(EO) ($\bullet$) and PTMA-poly(EO) ($\blacktriangle$). The final phase system contained (per kg): 4.32% (w/w) Dextran T-500; 3.59% (w/w) poly(EO) 6,000; 1.25 mmol sodium phosphate, pH 7.4; 4.75 mmol NaCl. Unsubstituted poly(EO) was replaced with an equal amount of poly(EO) derivative to give the indicated concentrations of terminal ligands. Substitution grades of PTMA-poly(EO), MA-poly(EO) and TMA-poly(EO) were calculated from elemental analysis to be 66%, 96% and 103%, respectively, where 100% substitution grade is two terminal molecules of ligand per linear poly(EO) molecule. We express the molarity of polymer-ligand as the ligand molarity, were it free in solution. Sufficient iodinated toxin to occupy approximately 7.3% of the α -toxin binding sites was added to a membrane fraction purified by sucrose density gradient centrifugation²³. Phase systems containing membrane-bound ^{125}I -labelled α -toxin were mixed and placed on ice for 15 min and then centrifuged at 500g for 15 min at 5.5°C. Aliquots of top (a) and bottom (b) phases were collected and counted in a Nuclear Chicago gamma-counter.

Table 1 Effect of bisquaternary methonium compounds on toxin binding and affinity partitioning

	Concentration (M) that inhibits initial rate of toxin binding by 50%	Concentration (M) that inhibits PTMA-poly(EO) effect by 50%
Decamethonium	3×10^{-8}	2×10^{-7}
Hexamethonium	1×10^{-6}	3×10^{-6}
Trimethonium	7×10^{-5}	$> 5 \times 10^{-4}$

Initial rate of ^{125}I -labelled *Naja naja siamensis* α -toxin binding was determined by a modification of the method of Schmidt and Raftery²⁶. Reaction mixtures contained 4×10^{-8} M acetylcholine receptor and fourfold molar excess of ^{125}I -labelled *Naja naja siamensis* α -toxin ($5\text{--}10$ Ci mmol⁻¹) in 10 mM phosphate, pH 7.4. The reaction rate approximated pseudo first-order kinetics. During incubation in the absence of inhibitory ligand the reaction progressed to 30–40% of completion and initial rates were calculated assuming a first order approach to equilibrium. The concentrations of bisquaternary methonium compounds required to inhibit the affinity partitioning of membrane-bound ^{125}I -labelled α -toxin in the top phase was determined using a system like that in Fig. 1 with 1.85×10^{-4} M PTMA-poly(EO).

receptors, or $\text{Na}^+\text{--K}^+$ -stimulated adenosine triphosphatase, or membrane-bound acetylcholinesterase can be fractionated from homogenates of the electric organ of *Torpedo californica*.

A standard two-phase system containing dextran and polyethylene oxide was used. The polymer-ligand, α,ω -bis-4-trimethylammonium (phenylamino) polyethylene oxide [PTMA-poly(EO)] was synthesised from (4-aminophenyl) trimethylammonium hydrochloride iodide (Eastman) and α,ω -dibromopolyethylene oxide by a method based on that described by Johansson²⁴. The quaternary ligand we used has been coupled to Sepharose through a side arm and used for affinity chromatographic purification of acetylcholinesterase and detergent-solubilised receptor²⁵. Since this compound like most others known to react with the cholinergic receptor is charged, other cationic ligand-polymer conjugates with anticipated differences in receptor affinity, α,ω -bis-trimethylammonium polyethylene oxide [TMA-poly(EO)] and α,ω -bis-methylamino polyethylene oxide [MA-poly(EO)] were examined for comparative purposes. At the same level of substitution, the conjugates would impart the same charge to the phase system.

Initial studies were conducted with electroplax membranes partially purified by density gradient procedures²³. Cholinergic receptor concentrations in the individual phases were monitored with ^{125}I -labelled *Naja naja siamensis* α -toxin ($5\text{--}10$ Ci per mmol) using the assay procedure of Schmidt and Raftery²⁶. This assay, and a simpler monitoring procedure that consists

of addition of toxin at concentrations less than 7.5% of site saturation before phase separation, yielded similar values for the partitioning of receptor between the two phases.

In a conventional aqueous polymer two-phase system (Fig. 1) less than 1% of membranes containing the cholinergic receptor was found in the polyethylene-oxide-rich top phase and about 65% was in the dextran-rich bottom phase. The remaining material was presumably localised to the interphase. When PTMA-poly(EO) was added there was a striking increase in the concentration of the membranes containing receptor in the top phase and a commensurate reduction in the bottom phase (Fig. 1). Addition of TMA-poly(EO) produced similar results although this polymer-ligand was somewhat less potent than PTMA-poly(EO) in this and other studies (Fig. 1). When MA-poly(EO) was used there was only a slight effect on the partitioning of the cholinergic-receptor-containing membranes even when relatively high concentrations were used (Fig. 1). Since MA-poly(EO) is a secondary amine which is about 99% charged at the pH used in these studies (7.4), the addition of a cationic polymer-ligand, which might be expected to alter the E.M.F. between phases²⁷, is not responsible for the partitioning effect that we observed.

To examine further whether the effect of PTMA-poly(EO) on the partitioning of membranes containing the cholinergic receptor was the result of specific binding to the cholinergic receptor site, we studied the effects of three bisquaternary methonium compounds with differing affinities for the cholinergic receptor. The affinity of these compounds for the cholinergic receptor was measured from inhibition of the initial rate of binding of ^{125}I -labelled α -toxin to membrane-bound receptor (Table 1). As expected, decamethonium was more potent than hexamethonium, which in turn was more potent than trimethonium. Addition of each of these compounds to the aqueous polymer two-phase system in the presence of PTMA-poly(EO) inhibited the affinity partitioning (Table 1) with an order of potency related to their affinity for a ligand-binding site on the receptor. We also determined the effect of progressive saturation of the cholinergic receptor with increasing concentrations of α -toxin (unlabelled) on the affinity partitioning effect of PTMA-poly(EO) in direct proportion to the saturation of toxin binding sites. Binding of toxin is known to compete with ligands behaving as agonists and antagonists for the nicotinic receptor²².

Having established that affinity partitioning could be applied with partially purified membranes, we investigated whether it could be used to purify membranes containing the cholinergic receptor from a crude mixture of membranes obtained from a homogenate of electroplax. When this mixture was added to a system containing 1.85×10^{-4} M PTMA-poly(EO), there was about a sevenfold increase in the specific activity of toxin binding in the membranes that partitioned to the top phase, as compared with the crude particulate fraction (Table 2). About 25% of the toxin binding activity was recovered in the top phase. The specific activity of ATPase in the membranes of the top phase was less than 5% of that of the crude particulate fraction and the specific activity of acetylcholinesterase in the membranes of the top phase was about 20% of that of the starting extract. It should be emphasised that all these activities are membrane bound since they were collected by centrifugation after the partitioning was completed. Partitioning of these membranes in an identical aqueous polymer two-phase system in the absence of PTMA-poly(EO) showed that the top phase contained less than 2% of the toxin binding activity, acetylcholinesterase or ATPase added to the system.

The purification achieved with a single partitioning step is comparable with that achieved by sucrose density gradient fractionation²³, since about 25% of the total protein in the membranes found in the top phase after affinity partitioning was found to be cholinergic receptor protein. Experiments in progress suggest that even greater purification can be achieved

Table 2 Specific activity of fractions from single extractions in phase system containing PTMA-poly(EO)

	ATPase*	AChE†	Toxin binding‡
Crude pellet	62.8	35	353
Bottom phase	26.4	20	201
Top phase	2.5	7	2,499

The phase system used in this extraction contained (per kg): 4.32% (w/w) Dextran T-500, 3.59% (w/w) poly(EO) 6,000; 1.85×10^{-4} M PTMA-poly(EO); 24.75 mmol NaCl; 4.75 mmol sodium phosphate, pH 7.4. A particulate fraction was obtained from a homogenate of electroplax by differential centrifugation¹⁸. This fraction, containing 15.6 mg protein was resuspended in 2 ml of 1 mM phosphate buffer, pH 7.4, and added to the phase system to give the above concentrations in 40 g of total system. The mixture was placed on ice, mixed by repeatedly inverting the tube, and then added to a 60-ml syringe fitted with a valve. The syringe assembly was centrifuged at 1,000 r.p.m. for 30 min at 5.5°C in a GLC-1 centrifuge (Sorvall). The top phase was collected directly and the bottom phase was collected through the valve. The top and bottom phases were diluted with distilled water and centrifuged for 3 h at 35,000 r.p.m. in a type 35 rotor (Beckman Instruments). Of the protein added to the phase system, 6.63 mg was recovered in bottom phase and 0.51 mg was recovered in top phase. No determination was made of the constituents in the interphase.

* μmol of ATP hydrolysed per h per mg protein²⁸.

† μmol of acetylcholine hydrolysed per min per mg protein²⁹.

‡ μmol of ^{125}I -labelled *Naja naja siamensis* toxin bound per mg protein²⁶.

either by sequential affinity partitioning steps or by combining the sucrose gradient centrifugation purification procedure with affinity partitioning.

These results suggest that a similar approach might be applied to the purification of cell membranes or even intact cells that contain sufficient concentrations of specific receptors. Preparative scale purifications should be possible since large volumes of two-phase systems can be handled conveniently.

This work was supported by US Public Health Service grants to P.T. and S.H.B. S.D.F. was supported by a predoctoral fellowship through a grant from the Alfred P. Sloan Foundation. We thank Dr James Patrick (Dartmouth) for purified toxin, Dr Jon Lindstrom (Salk Institute) for instructions on the iodination procedure, and Dr G. Johansson (Umea) for α, ω -bromopolyethylene oxide and TMA-poly(EO).

STEVEN D. FLANAGAN*

PALMER TAYLOR

SAMUEL H. BARONDES

Departments of Biology, Medicine and Psychiatry
University of California, San Diego

School of Medicine,
La Jolla, California 92037

Received January 17, 1975.

*Present address Friedrich Miescher-Institut, Basle, Switzerland.

- 1 Cuatrecasas, P., and Anfinsen, C. B., *A. Rev. Biochem.*, **40**, 259-278 (1971).
- 2 Wiggall, H., and Anderson, B., *J. exp. Med.*, **129**, 23-36 (1969).
- 3 Wiggall, H., and Mäkelä, O., *J. exp. Med.*, **132**, 110-126 (1970).
- 4 Wiggall, H., *Transplant. Rev.*, **5**, 76-104 (1970).
- 5 Evans, M. W., Mage, M. G., and Petersen, E. A., *J. Immun.*, **102**, 899-907 (1969); *ibid.*, **102**, 908-910 (1969).
- 6 Truffa-Bachi, P., and Wofsy, L., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 685-692 (1970).
- 7 Wofsy, L., Kumura, J., and Truffa-Bachi, P., *J. Immun.*, **107**, 725-729 (1971).
- 8 Edelman, G. M., Rutishauser, U., and Millette, C. F., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2153-2157 (1971); *ibid.*, **69**, 1596-1600 (1972).
- 9 Rutishauser, U., d'Eustacio, P., and Edelman, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3894-3898 (1973).
- 10 Choi, T. K., Sleight, D. R., and Nisonoff, S., *J. exp. Med.*, **139**, 761-766 (1974).
- 11 Rutishauser, U., and Sachs, L., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2456-2460 (1974).
- 12 Soderman, D. D., Germeshauser, J., and Katzen, H. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 792-796 (1973).
- 13 Melmon, K. L., Bourne, H. R., Weinstein, Y., and Sela, M., *Science*, **177**, 707-709 (1972).
- 14 Weinstein, Y., Melmon, K. L., Bourne, H. R., and Sela, M., *J. clin. Invest.*, **52**, 1349-1361 (1973).
- 15 Zachowski, A., and Parof, A., *Biochem. Res. Comm.*, **57**, 787-792 (1974).
- 16 Flanagan, S. D., and Baronides, S. H., *J. biol. Chem.*, **250**, 1484-1489 (1975).
- 17 Albertsson, P. Å., *Partition of cell particles and macromolecules*, second ed. (Almqvist and Wiksell, Stockholm, 1971).
- 18 Walter, H., *Methods in enzymology*, **32** (Academic, New York, in the press).
- 19 Albertsson, P. Å., *Methods in enzymology*, **31**, 761-769 (Academic, New York, 1974).
- 20 Nickel, E., and Potter, L. T., *Brain Res.*, **57**, 508-517 (1973).
- 21 Cartaud, J., Benedetti, L., Cohen, J. B., Meunier, J. C., and Changeux, J.-P., *FEBS Lett.*, **33**, 109-113 (1973).
- 22 Weber, M., and Changeux, J.-P., *Molec. Pharmacol.*, **10**, 1-40 (1974).
- 23 Duguid, J. R., and Raftery, M. A., *Biochemistry*, **12**, 3593-3597 (1973).
- 24 Johansson, G., Hartman, A., and Albertsson, P. Å., *Eur. J. Biochem.*, **33**, 379-386 (1973).
- 25 Chang, H. W., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2113-2117 (1974).
- 26 Schmidt, J., and Raftery, M. A., *Anal. Biochem.*, **52**, 349-354 (1973).
- 27 Reithman, R., Flanagan, S. D., and Baronides, S. H., *Biochim. biophys. Acta*, **29**, 193-202 (1973).
- 28 Avruch, J., and Wallach, D. F. H., *Biochim. biophys. Acta*, **233**, 334-347 (1971).
- 29 Reed, J., Goto, K., and Wang, C., *Anal. Biochem.*, **16**, 59-64 (1966).

Uptake of noradrenaline by an adrenergic clone of neuroblastoma cells

THERE are specialised transport systems mediating noradrenaline (NA) uptake from the extracellular fluid in various types of neurones in peripheral and central noradrenergic nerve terminals.

A specific and saturable high affinity uptake for the NA precursor tyrosine has been demonstrated in the adrenergic neuroblastoma clone NIE-115 (ref. 1). Another adrenergic neuroblastoma clone, M1 (ref. 2) possesses a transport system for tyrosine with similar characteristics. Cells of the M1 clone can synthesise ^3H -NA from ^3H -tyrosine; the ^3H -NA formed is found not only in the cells, but also in the medium where it attains a higher specific activity than in the cell interior (J.Z., unpublished). Thus a system may be operating which permits the passage of NA across the membrane. Here, we present data

which demonstrate the existence of an uptake for NA itself in the adrenergic neuroblastoma clone M1.

Cells of the adrenergic clone M1 isolated from C-1300 neuroblastoma were cultivated in Dulbecco's modified Eagle's MEM with foetal calf serum under an atmosphere of air- CO_2 at 37°C . Assays were performed in a phosphate-buffered saline medium (PBS) containing labelled NA (Fig. 1). The effect of amphetamine, desipramine, imipramine and ouabain on NA uptake was tested in the same medium.

When the initial velocities of $\text{L-}^3\text{H}$ -NA uptake by the neuroblastoma cells were plotted³ against various amine concentrations, the presence of two components was revealed (Fig. 1). One component operating at low NA concentrations has an apparent K_{m1} of $0.14\ \mu\text{M}$. This value for the high affinity transport of NA is similar to that reported for the isolated heart⁴ and synaptosomal preparations⁵⁻⁷. Another component which corresponds to a low affinity system has an apparent K_{m2} of $2.10^{-5}\ \text{M}$. This K_{m2} value was determined by incubation in NA concentrations (3×10^{-7} – $5 \times 10^{-4}\ \text{M}$) higher than indicated in Fig. 1. It is possible that this system is related to a non-saturable component. Free diffusion, however, cannot totally account for this NA influx, in view of the data in Table 1 which indicate that the low-affinity system is partially energy dependent.

The uptake of NA was inhibited when a metabolic poison 2,4-dinitrophenol (DNP) was added to the incubation medium in high concentrations (2 mM) 30 min before the addition of

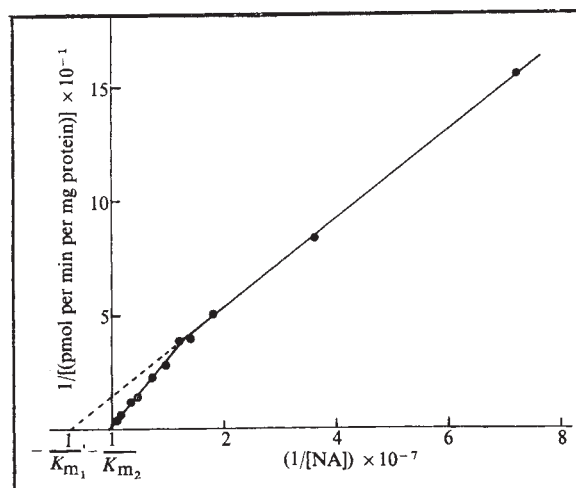


Fig. 1 Double reciprocal plot of $\text{L-}^3\text{H}$ -NA uptake into adrenergic clone M1 against NA concentrations. Cells were grown to confluency (4 d) in Petri dishes in Dulbecco's modified Eagle's MEM with 10% foetal calf serum under an atmosphere of 5% CO_2 in air at 37°C . Assays were performed at 37°C in 5 ml of phosphate buffered saline (PBS) containing: 137 mM NaCl; 2.6 mM KCl; 0.7 mM CaCl_2 ; 0.5 mM MgCl_2 ; 3.2 mM Na_2HPO_4 ; 1.4 mM KH_2PO_4 ; 10 mM glucose. The pH was adjusted to 7.3 (at 37°C) by addition of NaOH. The osmolarity was 305 mosmol l^{-1} . This medium was not found to have any detrimental effect on cell viability as judged by Trypan blue colouration. Ten minutes after replacement of the growth medium by PBS, the reaction was started by adding $\text{L-}^3\text{H}$ -NA (the Radiochemical Centre, Amersham; specific activity $7.2\ \text{Ci mmol}^{-1}$). Substrate was present in the reaction mixture at concentrations from 1.4×10^{-6} to $10^{-6}\ \text{M}$. The length of incubation was 2 min; preliminary experiments had shown that the uptake was linear for this time period. Reactions were terminated by removal of the incubation medium followed by four washes with NaCl 9‰ at 37°C . Cells were quantitatively harvested with 1 ml and one wash of 0.8 ml of distilled water. Then, 0.2 ml NaOH N were added. An aliquot (1.5 ml) of this solution was counted in the presence of 10 ml scintillation mixture (Monophase 40, Packard) in an Inter-technique SL-30 liquid scintillation spectrometer. Protein was determined by the method of Lowry *et al.*¹⁸ using bovine serum albumin as standard. Each point is the average of four determinations. Velocities are expressed in $10^{-12}\ \text{mol } ^3\text{H-NA per min per mg protein}$. The following kinetic constants were determined using the method of Wilkinson¹⁹. High affinity transport: apparent $K_{m1} = 0.14\ \mu\text{M}$; apparent $V_{max1} = 0.07\ \text{pmol per min per mg protein}$. Low affinity transport: apparent $K_{m2} = 2.10^{-5}\ \text{M}$; apparent $V_{max2} = 5.4\ \text{pmol per min per mg protein}$.

Table 1 Effect of various drugs and Na⁺ deprivation on NA uptake

	(NA) 1.4×10^{-8} M	(NA) 2×10^{-5} M
Control	100 ± 18 (12)	100 ± 17 (4)
DNP 2 mM	73 ± 10* (4)	69 ± 8* (4)
4 °C	36 ± 7‡ (4)	17 ± 7‡ (4)
Sucrose	53 ± 2‡ (4)	72 ± 2* (3)
Ouabain 10^{-4} M	58 ± 2‡ (4)	101 ± 15 (4)
Amphetamine $5 \cdot 10^{-5}$ M	75 ± 4* (5)	113 ± 23 (4)
Desipramine 10^{-8} M	61 ± 5† (3)	101 ± 27 (4)
Imipramine 10^{-5} M	62 ± 6† (4)	126 ± 30 (4)

Cells were grown and assayed as described in Fig. 1. ³H-NA was added after a 15-min preincubation with the drugs, except for DNP (30 min) and the reaction was stopped after 5 min. Each determination was repeated as indicated. Data are expressed as percentages of control uptake ± s.d., determined as d.p.m. of ³H per mg protein in the dish. When Na⁺ was omitted, sucrose was added in the same osmolarity. Control values (100%) for uptake (d.p.m. of ³H per mg protein ± s.d.) were $3,884 \pm 706$ and $1,818 \pm 306$ for NA concentration of 1.4×10^{-8} M and 2×10^{-5} M respectively. Significance, as judged by Student's *t*-test is indicated as follows:

* *P* < 0.05
† *P* < 0.01
‡ *P* < 0.001

labelled substrate. Those conditions have been reported to inhibit NA uptake into synaptosomal preparations⁸.

In addition of being energy-dependent the accumulation of NA was found to be temperature-sensitive. At 4 °C, both components of the NA uptake were greatly reduced. The high affinity process seemed to be dependent on the presence of Na⁺, since it was greatly reduced by replacing the NaCl in the incubation medium by sucrose of the same osmolarity.

In similar conditions, addition of ouabain (10^{-4} M) inhibited the high affinity uptake only. The low affinity process was less markedly sodium-dependent and was found to be not ouabain-sensitive. These data are in good agreement with those of Iversen⁹.

Potent inhibitors of catecholamine uptake such as imipramine, desipramine^{4,10,11} or amphetamine^{12,13} all inhibited the uptake of NA, but only that by the high affinity process (Table 1). Amphetamine was the least effective drug. The inhibition, however, was less marked than that described by Baldessarini and Vogt⁷ for rat brain homogenates. This relatively weak inhibition cannot be explained by the destruction of NA in the incubation medium since in separate experiments, we have observed that after 5 min of incubation, it remains at least 80% of the initial NA level. Thus our observations are probably the result of a difference between the neuroblastoma cell membrane and the cell membranes studied by other authors^{4,10-13}.

The uptake of NA in the adrenergic clone M1 of C-1300 neuroblastoma seems to have similar properties as the neuronal uptake studied by Iversen^{4,9}. First, a saturable high affinity transport system was found which was partially energy dependent and temperature sensitive. This uptake seems to require Na⁺ and to be inhibited by ouabain, amphetamine, imipramine and desipramine. Second, the presence of a saturable low affinity transport system was demonstrated. This low affinity uptake system was also partially energy dependent and temperature sensitive, but was not inhibited by the drugs tested.

In many respects, neuroblastoma cells grown in culture may be used as model systems for nerve cells¹⁴⁻¹⁷. We have shown that this applies also for NA uptake into an adrenergic clone. The similarities between NA uptake operating in the cultured cells and those shown to exist in other systems are especially noteworthy. Most studies with NA uptake processes have been done with nerve ending preparations or with tissues containing adrenergic nerve terminals, whereas cultivated neuroblastoma cells are devoid of synapses. When moreover nerve terminals are present, the investigation of transmitter uptake by the neuronal membrane is complicated by the presence of a second uptake into the storage granules. Adrenergic neuroblastoma clones may therefore represent a useful system for the study of catecholamine transport processes across the neuronal membrane.

We thank Dr C. Goridis for help with the manuscript, and Miss G. Ulrich for technical assistance.

J. ZWILLER
J. TRESKA-CIESIELSKI
G. MACK
P. MANDEL

Centre de Neurochimie,
Faculté de Médecine,
11, rue Humann,
67085 Strasbourg Cedex, France

Received December 30, 1974; revised February 11, 1975.

- Richelson, E., and Thompson, E. J., *Nature new Biol.*, **241**, 201-204 (1973).
- Mandel, P., Treska-Ciesielski, J., Hermetet, J. C., Zwiller, J., Mack, G., and Goridis, C., in *Frontiers in Catecholamine Research* (edit. by Usdin, E., and Snyder, S.), 277-283 (Pergamon, Oxford, 1973).
- Lineweaver, C. and Burk, D., *J. Am. chem. Soc.*, **56**, 658-666 (1934).
- Iversen, L. L., *The Uptake and Storage of Noradrenaline in Sympathetic Nerves* (Cambridge University Press, London, 1967).
- Snyder, S. H., and Coyle, J. T., *J. pharmac. exp. Ther.*, **165**, 78-86 (1969).
- Horn, A. S., Coyle, J. T., and Snyder, S. H., *Molec. Pharmacol.*, **7**, 66-80 (1971).
- Baldessarini, R. J., and Vogt, M., *J. Neurochem.*, **18**, 2519-2533 (1971).
- White, T. D., and Keen, P., *Biochim. biophys. Acta*, **196**, 285-295 (1970).
- Iversen, L. L., *Biochem. Pharmacol.*, **23**, 1927-1935 (1974).
- Iversen, L. L., *J. pharm. Pharmacol.*, **17**, 62-66 (1965).
- Squires, R. F., *J. pharm. Pharmacol.*, **26**, 364-366 (1974).
- Azzaro, A. J., Ziance, R. J., and Rutledge, C. O., *J. pharmac. exp. Ther.*, **189**, 110-118 (1974).
- Wenger, G. R., and Rutledge, C. O., *J. pharmac. exp. Ther.*, **189**, 725-732 (1974).
- Catterall, W. A., and Nirenberg, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3759-3763 (1973).
- Blume, A. J., Dalton, C., and Sheppard, H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3099-3102 (1973).
- Prasad, K. N., and Kumar, S., *Proc. Soc. exp. Biol. Med.*, **142**, 406-409 (1973).
- Prasad, K. N., Gilmer, K. N., and Sahu, S. K., *Nature*, **249**, 765-767 (1974).
- Lowry, O., Rosebrough, N. F., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265-275 (1951).
- Wilkinson, G. N., *Biochem. J.*, **80**, 324-332 (1961).

Mode of action of an anti-inflammatory fraction from normal human plasma

A FRACTION isolated from normal human plasma¹, contains a substance of low molecular weight (< 500) which shows anti-inflammatory activity in a number of animal models, including paw oedema tests in the mouse and rat^{1,2}, adjuvant arthritis in the rat³ and Arthus reactions in the rat and rabbit⁴. Its activity in the carrageenin-induced paw oedema test in the rat does not involve an interference with either the release or action of chemical mediators of inflammation such as histamine, 5-hydroxytryptamine, kinins or prostaglandins^{5,6}. The fraction is not active in inflammatory reactions in which these mediators play the more prominent role, for example, passive cutaneous anaphylaxis and the extravasation of plasma protein elicited by intradermal challenge with the mediators⁴, but shows marked and reproducible anti-inflammatory activity in situations in which the emigration of circulating leukocytes is a major factor, for example, Arthus reactions and the accumulation of polymorphonuclear and mononuclear cells into pleural and other inflammatory exudates⁷. The plasma fraction causes a reduction of up to 90% in the migration of leukocytes into the exudates found in porous inert sponges implanted subdermally in the rat⁷.

An important group of substances thought to induce the directed migration of leukocytes from the blood through the tissue extravascular spaces are the chemotactic factors derived from the complement system⁸. Although chemotaxis is an extremely difficult phenomenon to prove by direct observation *in vivo* the circumstantial evidence for it is overwhelming⁹. One example is the inhibition of migration of leukocytes into implanted sponge exudates in the rat when the circulating complement levels are depleted by treatment with purified cobra venom¹⁰. The plasma fraction does not act by depleting serum complement levels *in vivo* since it has no effect on the circulating titre of total haemolytic complement in the rat. Neither does it prevent the activation of the classical or alternate pathways of complement in fresh guinea pig serum by either antigen-antibody complexes, heat-aggregated γ globulin or zymosan, as assessed by measuring the residual total haemolytic titre⁷.

The preparations of the plasma fraction used were fraction II (ref. 1) further purified by absorption on Sephadex A25 ion-exchange resin and elution with 5% (w/v) acetic acid and 0.45% (w/v) NaCl. The activity associated with the final peak showing absorption at 254 nm was collected and de-salted by passage through Sephadex G-25. Three separate bulk preparations were made and tested for their effects on the accumulation of total leukocytes in sponges implanted subdermally in the rat⁷. The results (Table 1) show that the preparations produced a significant reduction, approximately 60%, on the migration of the leukocytes into the sponge exudate. There is some variability in activity between different preparations of plasma fraction and our normal practice is to define an active fraction as one which reduces either the development of swelling in the carrageenin-induced paw oedema test² or the migration of polymorphonuclear leukocytes into the 5 h sponge exudate⁷ in the rat by at least 50%. When the plasma fraction was heated at 100 °C for 1 h with 1.5 M NH₄OH solution (inactivated plasma fraction) it failed to inhibit the migration of leukocytes into the 5 h sponge exudate.

The second set of experiments used the Boyden chamber technique¹¹ to investigate the directed migration of rat and human leukocytes *in vitro*. This procedure not only allows quantitative assessment of chemotaxis but also investigation of interference with either the release or the action of complement-derived chemotactic factors. Polystyrene, disposable chemotactic chambers (Adaps, Dedham, Massachusetts) and Millipore filters with 3 µm pore size were used, and the methods for the assembly, incubation and counting of the cells which had penetrated the filter were as described by Goetzl and Austen¹². The upper chambers contained the leukocyte suspensions and to the lower chambers were added mixtures of serum and zymosan, which had previously been incubated for 1 h at 37 °C to ensure release of chemotactic factors. Plasma fraction preparations, tested on the same day as in the sponge experiments (Table 1) were added, in amounts ranging from 0.1 to 0.005 ml, either to the serum-activator mixture, before or after incubation, or to the cell suspension in the upper chamber. Random motility of the leukocytes was also studied in the chemotactic chambers by omitting the incubation of the serum with zymosan. The results (Table 2) show that when the plasma fraction was added either to the cell suspension or to the serum-activator mixture after incubation there was no significant change in the chemotaxis of either the rat or human leukocytes. When the plasma fraction, but not the inactivated plasma fraction, however, was added to the serum before incubation with the zymosan, a significant reduction in the directed migration of the leukocytes was observed. This action of the plasma fraction on the release of the chemotactic factors is dose dependent. Thus in the rat leukocyte-serum system the action increased from 10% with 0.01 ml of plasma fraction to 36% with 0.1 ml. The human cell-serum system is more sensitive and an inhibition in excess of 80% occurred after the addition of 0.02 ml of the plasma fraction.

The failure of the plasma fraction to significantly alter the directed migration when added to the cell suspensions in the upper chambers suggested that the plasma fraction did not act

Table 2 Effects of plasma fraction preparations on the release of chemotactic factors from rat and human serum *in vitro*

Addition to serum (lower chamber)	Addition to cells (upper chamber)	Number of cells	
		Rat	Man
(a) Directed migration experiments in chemotactic chambers*			
Saline	None	2.0±1.2 (12)	1.9±0.8 (9)
Before incubation with zymosan			
Saline	None	14.5±3.4 (12)	23.5±0.9 (15)
Saline	PF (0.1 ml)	12.8±4.1 (9)	26.8±0.3 (6)
PF (0.1 ml)	None	9.3±2.5 (12)†	ND
PF (0.02 ml)	None	11.3±0.7 (9)‡	4.6±1.9 (15)†
PF (0.01 ml)	None	13.3±1.2 (9)	9.6±6.9 (9)‡
PF (0.005 ml)	None	ND	15.2±8.6 (9)‡
IPF (0.1 ml)	None	14.7±0.8 (9)	ND
After incubation with zymosan			
PF (0.1 ml)	None	13.1±2.9 (9)	24.4±1.0 (6)
(b) Random migration experiments in chemotactic chambers§			
Saline	Saline	10.0±0.7 (9)	5.1±0.6 (9)
PF (0.1 ml)	PF (0.1 ml)	9.8±0.6 (9)	5.2±0.1 (9)

*PF, plasma fraction; IPF, inactivated plasma fraction (see Table 1). Leukocyte suspension (1.0 ml) containing 3 to 5 × 10⁶ cells per ml of either rat or human leukocytes in upper chamber; corresponding serum (0.9 ml), zymosan (50 µg) and either saline, PF, IPF or mixtures of these to give a final volume of 1.0 ml. Results given as mean ± s.d. and expressed as number of cells per high power field; ten such fields were counted in each experiment and number of separate experiments given in brackets.

†Significant difference ($P < 0.01$) from results in which no plasma fraction was added to the serum-zymosan mixture before incubation, in which the plasma fraction was added to the serum-zymosan mixture after incubation, in which the plasma fraction was added to the cell suspension in the upper chamber and in which inactivated plasma fraction was used.

‡Significant difference ($P < 0.01$) from results in which no plasma fraction was added to the serum-zymosan mixture.

§Reaction mixtures as for directed migration experiments except that incubation with zymosan omitted and either saline or plasma fraction added to both chambers.

ND, not determined.

on the leukocyte cell surface to prevent chemotactic attraction. Further evidence that the plasma fraction did not affect leukocyte behaviour and function is provided by the results of the random migration experiments in the chemotactic chambers (Table 2) and from other work in which suspensions of rat and human leukocytes exposed to the plasma fraction showed no changes in the uptake of trypan blue¹³, the phagocytosis of either ink or zymosan granules¹⁴, random motility in capillary tubes¹⁵, adherence to glass and rosette formation¹⁶.

We conclude that the plasma fraction exerts a selective action on the release of chemotactic factors, for example, C3a and C5a (ref. 17), after activation of the complement cascade by the alternate pathway *in vitro*. This effect may be of some significance *in vivo* since the effective amounts of the plasma fraction used in the present work are comparable. In the sponge experiments (Table 1) 1 ml of the fraction was injected intravenously and may be assumed to have been diluted in the circulation by a factor of 10. In the chemotactic chamber experiments (Table 2) amounts of the fraction were diluted from 1–10 to 1–200 with either rat or human serum in the lower chamber. When complement proteins and leukocytes have entered inflammatory exudates a vicious circle is set up because leukocyte lysosomes not only contain a C5-cleaving enzyme¹⁷ but a substance causing a non-immune activation of the complement system¹⁸. More chemotactic fragments may be generated by the interaction of the leukocytes and extravascular complement thus amplifying the inflammatory response through positive feedback mechanisms¹⁸. The persistence of an inflammatory reaction may be largely dependent on the secondary events mediated by leukocytes which have accumulated at the site of the inflammatory insult. The anti-inflammatory activity of the plasma fraction in susceptible animal models, that is, those associated with neutrophil emigration such as carrageenin-induced paw oedema, Arthus

Table 1 Effects of plasma fraction preparations on leukocyte migration into the 5 h sponge exudate in the rat

Group	Number of cells*
Control	29.1 ± 5.9
Plasma fraction	13.0 ± 1.7†
Inactivated plasma fraction	29.5 ± 3.4

*Results given as mean ± s.d. and expressed as number of cells × 10⁴ per ml of sponge exudate⁷. One sponge implanted in each rat which received an intravenous injection of 1 ml of either saline, plasma fraction or inactivated plasma fraction; five animals per group; the experiments were carried out on three separate occasions.

†Significant difference ($P < 0.001$) from results of control and inactivated plasma fraction groups.

reactions and implanted sponges, is explicable on the basis of a selective interference with the release of complement-derived chemotactic fragments. This rather specific site of action may be of wider relevance since it has been proposed¹⁹ that a logical approach to anti-inflammatory therapy would be a blocking of any one of the many steps in the chemotactic response of the neutrophil.

We thank the National Research Development Corporation, the Wates Foundation and the King's College Hospital and Medical School Research Committee for financial support.

J. R. WALKER
M. J. H. SMITH
A. W. FORD-HUTCHINSON
F. J. BILLIMORIA

Department of Biochemical Pharmacology,
King's College Hospital Medical School,
Denmark Hill,
London SE8 5RX, UK

Received December 13, 1974; revised February 3, 1975.

- ¹ Ford-Hutchinson, A. W., Insley, M. Y., Elliott, P. N. C., Sturgess, E. A., and Smith, M. J. H., *J. Pharm. Pharmacol.*, **25**, 881-886 (1973).
- ² Smith, M. J. H., Colledge, A. J., Elliott, P. N. C., Bolam, J. P., and Ford-Hutchinson, A. W., *ibid.*, **26**, 836-837 (1974).
- ³ Elliott, P. N. C., Bolam, J. P., Ford-Hutchinson, A. W., and Smith, M. J. H., *ibid.*, **26**, 751-752 (1974).
- ⁴ Bolam, J. P., Ford-Hutchinson, A. W., Elliott, P. N. C., and Smith, M. J. H., *ibid.*, **26**, 660-661 (1974).
- ⁵ Bolam, J. P., Elliott, P. N. C., Ford-Hutchinson, A. W., and Smith, M. J. H., *ibid.*, **26**, 434-440 (1974).
- ⁶ Smith, M. J. H., Ford-Hutchinson, A. W., Elliott, P. N. C., and Bolam, J. P., *ibid.*, **26**, 692-698 (1974).
- ⁷ Ford-Hutchinson, A. W., *et al.*, *ibid.*, **27**, 106-112 (1975).
- ⁸ Nelson, R. A., in *The Inflammatory Process*, 3, second ed. (edit. by Zweifach, B. W., Grant, L., and McCluskey, R. T.), (Academic, New York, 1974).
- ⁹ Grant, L., *ibid.*, **2**.
- ¹⁰ Wiener, S., Lendvai, S., Rogers, B., Urvitsky, M., and Meilman, E., *Am. J. Pathol.*, **73**, 807-816 (1973).
- ¹¹ Boyden, S., *J. exp. Med.*, **115**, 453-466 (1962).
- ¹² Goetzl, E. J., and Austen, K. F., *J. exp. Med.*, **136**, 1564-1580 (1972).
- ¹³ Stevenson, R. D., *Clin. exp. Immunol.*, **14**, 417-426 (1973).
- ¹⁴ Böyum, A., *Scand. J. clin. Lab. Invest.*, **21**, suppl. 97, 77-89 (1968).
- ¹⁵ Maini, R. N., Roffe, L. M., Magrath, I. T., and Dumonde, D. C., *Int. Archs Allergy appl. Immunol.*, **45**, 308-321 (1973).
- ¹⁶ Durance, R. A., Micheli, A., and Fallet, G. H., *Ann. rheum. Dis.*, **33**, 216-229 (1974).
- ¹⁷ Vogt, W., *Pharmac. Rev.*, **26**, 125-169 (1974).
- ¹⁸ Goldstein, I. R., and Weissmann, G., *J. Immunol.*, **113**, 1583-1588 (1974).
- ¹⁹ Ward, P. A., in *Inflammation, Mechanisms and Control* (edit. by Lepow, I. H., and Ward, P. A.), (Academic, New York, 1972).

Electrophoretic variation in allelozymes related to function or structure?

ATTEMPTS to explain variation at enzyme loci in terms of the function of the protein molecule¹⁻⁵ have shown that enzymes characterised by a single physiological substrate are less variable than enzymes which have been shown *in vitro* to act on a number of substrates⁶ and that enzymes regulating the flux through a pathway are more variable than enzymes with no regulatory function^{3,4}. The explanation for this is that different enzymatic forms are favoured in different environments. What distinguishes this hypotheses from others concerning the maintenance of protein variation is that it is to a large extent independent of mutation rate, population size, gene flow among populations and whether or not the population is at equilibrium. For these reasons it can easily be tested. Johnson⁴ and Gillespie and Langley⁵ analysed separately data from *Drosophila*, small mammals and man. Although the collection of data on small mammals and man is more or less complete, both papers, and that by Gillespie and Langley⁵ in particular, ignored a number of *Drosophila* studies. I have collected electrophoretic data referring to 61 species and semispecies of *Drosophila* (Fig. 1) and carried out an analysis identical to that of Johnson⁴, supporting the observation that enzymes with multiple substrates are more variable than enzymes with single substrates. Also, enzymes with variable substrates are more polymorphic than regulatory enzymes, which in turn are more polymorphic than non-regulatory enzymes.

This analysis (see Fig. 1) does not take into account the distribution of variation within the classes mentioned above.

Figure 2 gives this distribution for the 260 hydrolases and for the 133 esterases in particular; 23% of the hydrolases and 21% of the esterases are monomorphic. In most of the studies cited in Fig. 1, several populations were examined and all were found to be identically monomorphic at a number of loci with esterase, phosphatase and peptidase activity^{6,11,16-18}. When closely-related species were studied it was found that this conservatism transcends the species limits^{6,22}. It seems that about one-fifth of the loci specifying enzymes with hydrolytic activity constitute some of the most conservative loci studied in *Drosophila*; yet they are in the class of multiple or variable substrates.

Both α glycerophosphate dehydrogenase (α GPDH) and malic dehydrogenase (MDH) are classified as non-regulatory and by implication their evolution has been guided by the same type of selection forces⁴. But α GPDH is consistently poorer in variation than MDH (see Fig. 1). Table 1 shows how non-regulatory enzymes change within three groups of closely related species. Within the *willistoni* group only two species share the same major allele at MDH, all the others being almost fixed for different alleles. No differentiation took place, however, at α GPDH. This marked difference repeats itself in the other two groups and it is obvious that when looked at over periods of millions of years, MDH and α GPDH have followed different evolutionary patterns.

The classification of enzyme loci according to their function evidently cannot account for the pattern of their variation, although a pattern may arise when heterogeneous data are pooled. One reason may be that the assignment of a locus to a

Table 1 Number of major alleles observed within a group of closely related species of *Drosophila*

Enzyme	Group		
	<i>willistoni</i> (6 species)	<i>obscura</i> (11 species)	<i>repleta</i> (12 species)
MDH	5	9	8
α GPDH	1	3	1
Aldolase	1	—	3
Fumarase	2	—	2
Isocitric dehydrogenase	1	6	—
Triose phosphate isomerase	3	4*	—

* Studied in ten species.

Data for the *willistoni* group from Ayala and Tracey^{2,3} and Ayala *et al.*²⁴, for the *obscura* group from Lakovaara and Saura²², and for the *repleta* group this study.

multiple substrate group is based on the *in vitro* behaviour of the enzyme, which may indicate nothing about its physiological role. This remains unknown for the majority of these non-specific enzymes. Contrary to their ability to utilise a wide range of substrates *in vitro*, their function *in vivo* may be quite specific. *EstA* of the olive fruit fly *Dacus oleae* behaves *in vitro* like an acetylcholinesterase²⁵; flies heterozygous for a 'null' allele are less resistant to organophosphate insecticides, and the enzyme is present in the head and the thorax, but not in the abdomen²⁶. The evidence, therefore, is convincing that *EstA* is associated with the nervous rather than the digestive system of the insect. If so, it is difficult to see to what kind of environmental variability this enzyme has been exposed. Yet, *EstA* is one of the most polymorphic loci described so far: more than fifteen alleles have been detected and in a population of *D. oleae* as much as 76% of the individuals are heterozygous²⁷.

Far more variation has been observed in some esterases and far less at α GPDH than in loci of other enzymatic functions. This observation can be explained by postulating that the most important single factor determining the extent of electrophoretic variation is the primary structure of the protein molecule. The primary structure determines the extent directly and not through the functional properties of the enzyme and the environmental diversity to which it is exposed. King²⁸ argued that, provided the size of the population is maintained above a

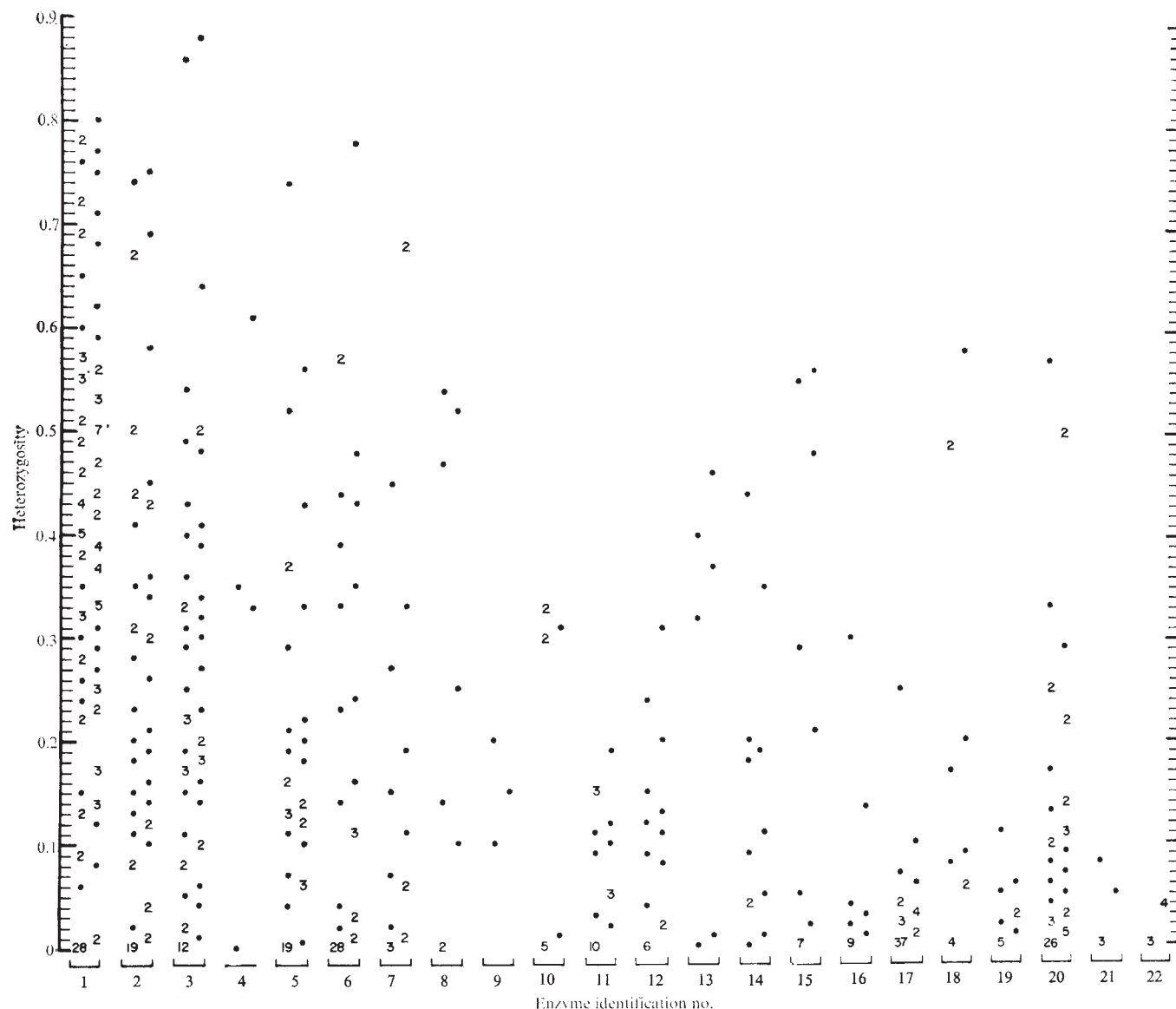


Fig. 1 Estimates (635) of heterozygosity at a number of *Drosophila* loci. The following species were included in the analysis: *affinis*², *aldrichi*⁸, *ananasae*⁷, *arizonensis*⁶, *athabasca*², *bipunctinata*⁸, *busckii*⁹, *equinoxialis*¹⁰, *malerkotliana*⁸, *melanogaster*², *mojavensis*⁶, *mulleri*⁸, *obscura*¹¹, *pachea*¹², *parabipunctinata*⁸, *paulistorum*¹³ semispecies *A*, *AB*, *I* and *T*, *pavani*¹⁴, *persimilis*¹⁵, *pseudoobscura*¹⁶, *robusta*¹⁷, *simulans*², *subobscura*¹⁸, *tropicalis*¹⁹, *willistoni*²⁰, and 34 species of the genus *Drosophila* in Rockwood *et al.*²¹. Identification numbers stand for: 1, esterase; 2, phosphatase; 3, peptidase; 4, amylase; 5, octanol dehydrogenase; 6, alcohol dehydrogenase; 7, aldehyde oxidase; 8, adenylate kinase; 9, glyceraldehyde-3-phosphate dehydrogenase; 10, glucose-6-phosphate dehydrogenase; 11, hexokinase; 12, malic enzyme; 13, phosphoglucose isomerase; 14, phosphoglucomutase; 15, xanthine dehydrogenase; 16, aldolase; 17, α GPDH; 18, isocitric dehydrogenase; 19, fumerase; 20, malate dehydrogenase; 21, 6-phosphogluconate dehydrogenase; 22, triose phosphate isomerase. Numbers in the body of the figure indicate the number of times the corresponding value of heterozygosity has been observed at a given enzyme locus; dots indicate this value has been observed once. The amount of variation at a given locus is taken to be independent of the amount of another locus of the same or other species. The multiple substrate group of Gillespie and Langley⁵ includes loci from No. 1 to No. 7. The single substrate group includes the rest of the loci. The Wilcoxon two-sample rank test performed on the heterozygosities at these loci gives $u_{1-p} = -7.82$. According to Johnson⁴, loci from No. 1 to No. 5 fall in the group variable substrate, loci from No. 6 to No. 15 in regulatory, and the rest in non-regulatory enzymes. Regulatory against variable substrate gives $u_{1-p} = -5.24$, and non-regulatory against regulatory, $u_{1-p} = -3.93$. α GPDH against MDH gives $u_{1-p} = -3.44$. In all tests (corrected for ties) $P < 0.001$. Loci from No. 1 to No. 4 constitute the class of hydrolases.

certain limit, the electrophoretic variation at a protein locus is determined by the number of lysine, arginine, glutamic, and aspartic acid residues and, to a lesser extent, by the probability that a conformational change will not affect the protein function. Given that this property remains basically unchanged and the composition in the four amino acids mentioned above varies little from species to species, we expected (and found^{16,19,24}) the same amount of polymorphism at homologous loci over populations of the same and related species. The differences we observe may be attributed to sampling error or

to genetic drift. Indeed, the heterozygosity distribution of esterases (Fig. 2), excluding the zero class, is close to normal, as would be expected if these 105 esterases were drawn from the same Universe. (When the expected numbers are calculated on a 10% heterozygosity interval, a test for normality gives: $\chi^2 = 4.79$; d.f. = 7; $0.60 < P < 0.70$.)

The hypothesis predicts that structurally related enzymes will show comparable amounts of variation. Hydrolases represent such a group. Excluding the zero class, the distribution of heterozygosities at the remaining 200 hydrolases does not

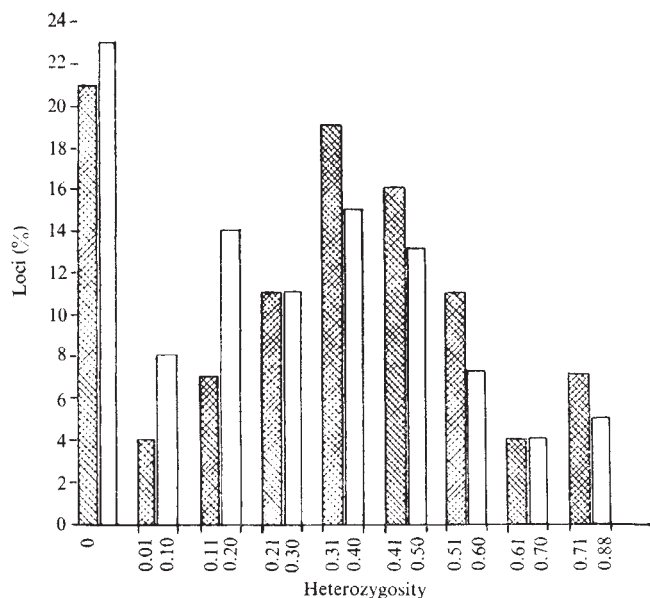


Fig. 2 Distribution of heterozygosities. Open bars, hydrolases; shaded bars, esterases.

deviate significantly from normal ($\chi^2=11.38$; d.f.=7; $0.10 < P < 0.20$). Hydrolases with zero heterozygosity may represent a separate class. It is possible that these conservative esterases, phosphates and peptides are closer to each other in terms of evolution than they are to esterases, phosphatases and peptidases which constitute the polymorphic fraction of hydrolases. Oppenoorth and van Asperen^{29,30} observed that a single mutation altered an aliesterase of the house fly into an enzyme with phosphatase activity. This observation demonstrates the possibility that enzymes of different function may be structurally much closer to each other than they are to enzymes with the same function, especially when this function is determined by a nonspecific substrate.

This explanation of electrophoretic polymorphism is compatible with the hypothesis of neutrality³¹, since it attaches no significance to the environmental variability. Both function and polymorphism are determined by the primary structure of the molecule, and thus seem to be correlated, although this correlation is not a causal one. The term allozymes, coined by Prakash *et al.*¹⁶ to denote the allelic forms of an enzyme locus, is not entirely proper. The greek *allos* for 'other' or 'foreign' does not imply relation. The proper word would be allelozymes.

I thank Professor M. R. Wheeler for providing me with species of the *repleta* group of *Drosophila* and Professor R. C. Lewontin for comments. This work was supported by a grant from the NRC of Canada.

E. ZOUIROS

Department of Biology,
Dalhousie University,
Halifax, Nova Scotia, Canada

Received October 7, 1974; revised January 17, 1975.

- Gillespie, J. H., and Kojima, K., *Proc. natn. Acad. Sci. U.S.A.*, **61**, 582-585 (1968).
- Kojima, K. J. H., Gillespie, and Tobari, Y. N., *Biochem. Genet.*, **4**, 627-637 (1970).
- Johnson, G. B., *Nature*, **232**, 347-349 (1971).
- Johnson, G. B., *Science*, **184**, 28-37 (1974).
- Gillespie, J. H., and Langley, C. H., *Genetics*, **76**, 837-848 (1974).
- Zouros, E., *Evolution*, **27**, 601-621 (1974).
- Johnson, F. M., *Genetics*, **68**, 77-95 (1971).
- Young, S., Wheeler, L., and Bock, I., *Studies in Genetics* (Univ. Texas Publ., No. 7213, 1971).
- Prakash, S., *Genetics*, **75**, 571-576 (1973).
- Ayala, F. J., Powell, J. R., and Tracey, M. L., *Genet. Res.*, **20**, 19-42 (1972).
- Lakovaara, S., and Saura, A., *Genetics*, **69**, 377-384 (1971).
- Rockwood, S., and Johnston, J. S., and Heed, W. B., *Genetics*, **73**, 135-146 (1973).
- Richmond, R. C., *Genetics*, **70**, 87-112 (1972).
- Kojima, K., Smouse, P., Young, S., Nair, P. S., and Brncic, D., *Genetics*, **72**, 721-731 (1972).
- Prakash, S., *Proc. natn. Acad. Sci. U.S.A.*, **62**, 778-784 (1969).
- Prakash, S., Lewontin, R. C., and Hubby, J. L., *Genetics*, **61**, 841-858 (1969).

- Prakash, S., *Genetics*, **75**, 347-369 (1973).
- Lakovaara, S., and Saura, A., *Hereditas*, **69**, 77-82 (1971).
- Ayala, F. J., *Proc. sixth Berkeley Symp. Math. Stat. Prob.*, **5**, 211-236 (1972).
- Ayala, F. J., Powell, J. R., Tracey, M. L., Mourao, C. A., and Perez-Salas, S., *Genetics*, **70**, 113-139 (1972).
- Rockwood, E. S., Kanapi, C. G., Wheeler, M. R., and Stone, W. S., *Studies in Genetics* (Univ. Texas Publ. No. 7103, 1971).
- Lakovaara, S., Saura, A., and Falk, C. T., *Evolution*, **26**, 177-184 (1972).
- Ayala, F. J., and Tracey, M. L., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 999-1003 (1974).
- Ayala, F. J., Tracey, M. L., Barr, G., MacDonald, J. F., and Perez-Salas, S., *Genetics*, **77**, 343-384 (1974).
- Zouros, E., Tsakas, S., and Krimbas, C. B., *Genet. Res.*, **12**, 1-9 (1968).
- Tsakas, S., and Krimbas, C. B., *Evolution*, **24**, 807-815 (1970).
- Zouros, E., and Krimbas, C. B., *Genet. Res.*, **14**, 249-258 (1969).
- King, J. L., *Genetics*, **76**, 607-613 (1974).
- Oppenoorth, F. J., and van Asperen, K., *Science*, **132**, 298-299 (1960).
- Oppenoorth, F. J., *A. Rev. Entomol.*, **10**, 185-206 (1965).
- Kimura, M., and Ohta, T., *Nature*, **229**, 467-469 (1971).

Biosynthesis of alkyl sulphides by an ant

ALKYL sulphides are relatively common in microorganisms and higher plants^{1,2}, but rare in animals. In the arthropods they seem to be restricted to the African stink ant *Paltothyreus tarsatus*^{3,4}, where they are produced in the mandibular glands and function as releasers of digging behaviour. The two sulphides produced by the ant are dimethyl disulphide and dimethyl trisulphide⁵. An investigation of the biosynthesis of these compounds seemed particularly interesting for several reasons: their *de novo* production in animals is unknown, they seem to be unique to *Paltothyreus* and biosynthetic studies of very few arthropod pheromones have been undertaken.

Although microorganisms and plants such as onions, chives and garlic are known to produce both dimethyl disulphide and dimethyl trisulphide^{1,6}, the biosynthesis of these compounds has not been extensively investigated. In plants dimethyl disulphide is known to be produced from methionine as a byproduct of ethylene production⁷, and in microorganisms⁸ and fungi⁹ by oxidative deamination and demethiolation of methionine. Since these biosynthetic studies suggested that it was the starting material for sulphide biosynthesis, methionine was chosen as the likely material from which *Paltothyreus* might synthesise its sulphides. Insects were previously known only to utilise methionine for trans-sulphuration to cysteine by way of cystathionine pathway¹⁰.

Paltothyreus workers fed on a diet of termites (*Macrotermes natalensis*) were injected with solutions containing L-³⁵S-methionine, DL-(1-¹⁴C)-methionine and L-(methyl-¹⁴C)-methionine. The ants were maintained in the laboratory for 4 d after

Table 1 Incorporation of the label from methionine into *Paltothyreus* sulphides

Labelled methionine	Activity administered (d.p.m.)	Activity in disulphide (d.p.m.)	Activity in trisulphide (d.p.m.)
L-(³⁵ S)-	2.5×10^6	1×10^4	5.7×10^4
DL-(1- ¹⁴ C)-	2.2×10^6	0	0
L-(methyl- ¹⁴ C)-	2.1×10^6	6.1×10^2	2.4×10^3

injection. They were then decapitated, their mandibular glands removed and extracted with methylene chloride. This extract, containing the sulphides, was fractionated using gas chromatography and the di- and tri-sulphides individually collected by preparative gas chromatography. The fractionated di- and tri-sulphides were then each placed in vials containing 15 ml of Packard Instagel scintillant and their radioactivity determined in a Beckman scintillation counter. Table 1 shows that *Paltothyreus* can utilise the sulphur in methionine for the production of the sulphides. In addition, the methyl groups in the sulphides seem to arise from the methyl group of the methionine since labelled ¹⁴C is incorporated into the sulphides only when it is in the terminal methyl group.

This work provides the first experimental evidence for the *de novo* biosynthesis of sulphides in an arthropod. The dimethyl disulphide is probably produced from methionine in much the

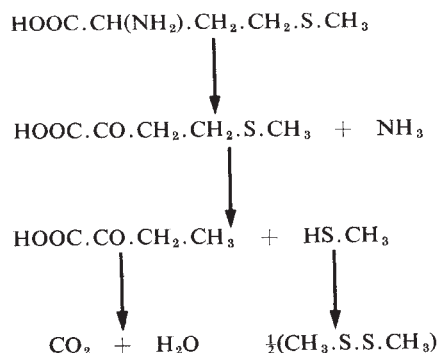


Fig. 1 Possible route for the biosynthesis of dimethyl disulphide from methionine.

same way (Fig. 1) as has been found in microorganisms^{8,9}. In the case of the trisulphide the situation is more complex, since even if the two methyl groups and their attached sulphur atoms come from methionine, the origin of the third sulphur atom remains unknown. To determine whether the methanethiol group from methionine is incorporated directly into both the disulphide and the trisulphide, further experiments using methionine doubly labelled with ³H and ³⁵S are being undertaken.

Whatever the uncertainties about the precise biosynthetic pathways utilised for sulphide production in *Paltothyreus*, it is clear that methionine can be utilised as the starting material for the biosynthesis of both exocrine products.

This work was supported by a grant from the South African Atomic Energy Board.

R. M. CREWE

Department of Entomology,
University of Natal,
Pietermaritzburg 3200

F. P. ROSS

Department of Physiological Chemistry,
Medical School,
University of the Witwatersrand,
Johannesburg, South Africa

Received January 20, 1975.

- ¹ Kadota, H., and Ishida, Y., *A. Rev. Microbiol.*, **26**, 127-138 (1972).
- ² Pandeya, S. N., *J. scient. ind. Res.*, **31**, 320-331 (1972).
- ³ Blum, M. S., *Proc. seventh Congr. I.USSI, Lond.*, 23-40 (1973).
- ⁴ Crewe, R. M., and Fletcher, D. J. C., *J. ent. Soc. sth. Afr.*, **37**, 291-298 (1974).
- ⁵ Casnati, G., Ricca, A., and Pavan, M., *Chimica Ind., Milano*, **49**, 57-58 (1967).
- ⁶ Bernhard, R. A., *Phytochemistry*, **9**, 2019-2127 (1970).
- ⁷ Luckner, M., *Secondary Metabolism in Plants and Animals*, 236 (Chapman & Hall, London, 1972).
- ⁸ Segal, W., and Starkey, R. L., *J. Bact.*, **98**, 908-913 (1969).
- ⁹ Ruiz-Herrera, J., and Starkey, R. L., *J. Bact.*, **99**, 544-551 (1969).
- ¹⁰ Leckstein, P. M., *Comp. Biochem. Physiol.*, **49B**, 743-747 (1974).

Subunit structure of chromatin is the same in plants and animals

ALTHOUGH plants and animals diverged from each other nearly 10⁹ yr ago, the histone composition of their chromosomes is remarkably similar¹; in particular, the arginine-rich histones III and IV of peas and cows differ by only four and two amino acids, respectively^{2,3}. Such extreme evolutionary conservation of a protein sequence argues that histones are crucial to chromosome structure and/or function, and further suggests considerable similarity, at the molecular level, between the chromosomes of plants and animals. We present evidence here for such a similarity.

In animals, chromatin seems to be organised into subunit structures, involving several hundred base pairs of DNA and small numbers of each class of histones⁴⁻⁸. Important evidence for a model of this type, in which subunits are arranged as beads on a string, is that, after nuclease digestion of the chromatin, most chromosomal DNA can be isolated as pro-

ected fragments which are integral multiples of a monomeric fragment, 100-200 base pairs long⁵⁻⁸. We have done similar nuclease digestions of plant chromatin and have obtained similar results.

Chromatin was isolated from pea seedlings (*Pisum sativum*) by a simplification of the method of Bonner *et al.*⁹, using a low ionic strength buffer previously shown to inhibit histone migration⁷. After incubation of the crude chromatin with various concentrations of micrococcal nuclease, the remaining DNA was purified and subjected to agarose gel electrophoresis as described in the legend to Fig. 1. As Fig. 1d shows, when pea seedling chromatin was treated exhaustively with nuclease, a discrete DNA fragment remained intact (even when free radioactive DNA or RNA added to the digestion mixture was

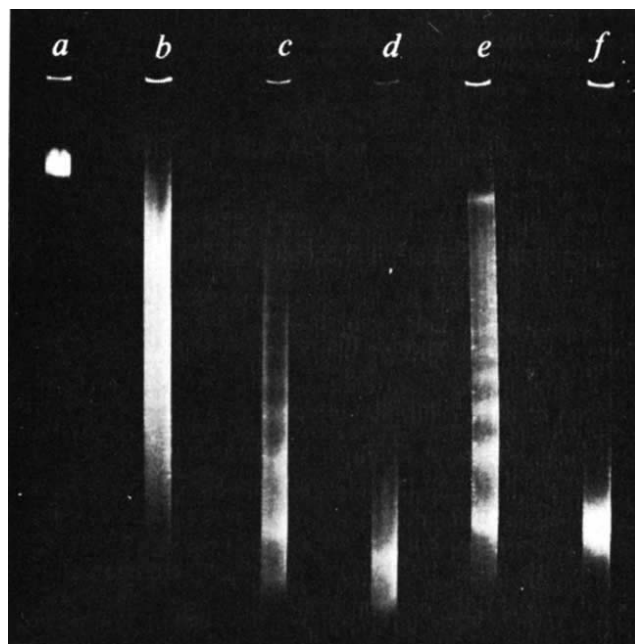


Fig. 1 Agarose gel electrophoresis of: λ DNA (a); DNA purified from crude pea chromatin after incubation with 0, 10, and 100 $\mu\text{g ml}^{-1}$ of micrococcal nuclease, respectively, as described below (b, c and d); DNA isolated from a partial digest of mouse myeloma chromatin (e); peak fraction from a sucrose gradient of DNA purified from extensively digested mouse myeloma chromatin (and run as a standard in all sets of gels) (f). Gels were prepared from 1.4% agarose and run in a solvent of 0.04 M Tris, 10⁻³ M EDTA, pH 7.8, plus 0.1% ethidium bromide¹³. The dye, when intercalated into the DNA, fluoresces when the gel is examined under ultraviolet light. Pea seeds were germinated in the dark, and the shoots collected, washed, frozen, ground with 2 volumes of cold buffer (5 $\times$ 10⁻³ M sodium phosphate, 2.5 $\times$ 10⁻⁵ M CaCl₂, pH 7.0)⁷ and blended for 60 s. The extract was filtered through eight layers of cheesecloth and centrifuged at 4000g for 30 min at 0-4 °C. The crude chromatin pellet was removed from the underlying starch pellet and resuspended in 1/50 to 1/100 of the original volume of buffer. Micrococcal nuclease (Worthington) was added to various concentrations and incubated at 37 °C. After 20 min, ribonuclease was added to 0.2 mg ml⁻¹, and at 30 min EDTA, sodium dodecyl sulphate and nuclease-free pronase were added to final concentrations of 10⁻³ M, 0.1% and 0.5 mg ml⁻¹ respectively. The mixtures were incubated a further 30 min at 37 °C and then extracted two or three times with phenol. Phenol was removed either by ether extraction followed by bubbling with nitrogen, or by passage twice through a short column of P-2 (BioRad). The bands in the gels disappeared on incubation with DNase I, DNase II and micrococcal nuclease, but not with RNase, pronase, α -amylase or cellulase. Thermal denaturation indicated that the purified DNA was native, with a base composition close to that of pea seedling DNA. The same digestion patterns were seen with several minor variations of the above protocol; for example, using a lower ionic strength buffer (10⁻³ M Tris, 2.5 $\times$ 10⁻⁵ M CaCl₂, pH 8.4), extracting with a Dounce homogeniser rather than blending, or adding 1-2 mg ml⁻¹ of α -amylase at the same time as the ribonuclease. Similar digestion patterns have been obtained with chromatin isolated from the shoots of black-eyed peas (*Vigna sinensis*, unpublished).

rendered greater than 95% acid soluble). Comparison of the amount of DNA in Fig. 1d with that of the no-enzyme control (Fig. 1b) indicates that more than half of the isolated DNA migrated as this protected fragment. Moreover, the size of this fragment, as estimated from the distance migrated in the gel, was close to that of a DNA fragment obtained from a digest of nuclei isolated from mouse myeloma cells (Fig. 1f; using a method similar to ref. 6; J.D.M. and Kimmel, C. B., unpublished). With less extensive nuclease treatment of the pea chromatin, a series of DNA bands (at least seven) was obtained (Fig. 1c), corresponding closely to DNA bands seen in a partial digest of either mouse nuclei (Fig. 1e) or calf thymus chromatin (kindly provided for comparison by B. R. Shaw and K. E. Van Holde). When the logarithm of the band number (counting back from the fastest running band) is plotted against the distance migrated in the gel (Fig. 2), the plot is linear, indicating that the length of the DNA in any particular band is an integral multiple of the length of the DNA in the monomer band. Furthermore, the data from pea, calf (not shown) and mouse digests fall on close, parallel lines, indicating similar fragment sizes. From the differences of the intercepts of least squares lines fitted to such

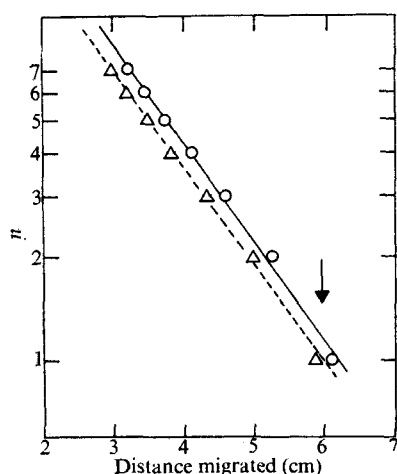


Fig. 2 Plot of the logarithm of the band number (n) against distance migrated in the gel: ○, pea chromatin digest; △, mouse myeloma chromatin digest. Distances were averaged from four to nine densitometer traces of film negatives of the gels (varying gel position, film exposure times and so on), and each point is the average of three parallel gels. The arrow indicates the migration position of the sucrose gradient fraction from a mouse digest used in Fig. 1f, above, which has been shown to be 170 ± 30 base pairs, as measured by sedimentation velocity. The lines represent the least-squares best fit of the data to the equation: $\log(n) = a(\text{distance migrated}) + b - \log(\text{size of monomer fragment})$.

data, the ratio of the length of the monomer DNA fragment in pea seedling chromatin to that in mouse chromatin can be estimated as 0.95 ± 0.2 (weighted average from 14 gels). We estimate the absolute size of the monomer DNA fragment as 170 ± 30 base pairs, based on the gel migration of a purified mouse myeloma DNA fraction (Fig. 1f) which had a sedimentation coefficient ($S_{0,w}^0$) of 5.5 ± 0.3 as determined by analytical band sedimentation^{10,11}. This size estimate lies between those previously made on DNA fragments isolated from rat liver⁶ and calf thymus chromatin^{5,7}. Although we have not seen a discrete submonomer band (as has been described by Noll⁶ in a digest of rat liver chromatin), as Fig. 1c shows, some DNA migrated ahead of the monomer band. Furthermore, after prolonged nuclease treatment (for example, Fig. 1d) most of the DNA migrated to a position in the gel corresponding to a size 80% of the monomer band, or about 140 base pairs (the $S_{0,w}^0$ of the DNA sample used in Fig. 1d was 5.0 ± 0.3 , corresponding to a DNA length of 125 ± 20 base pairs).

As with similar studies on mammalian systems, the simplest model to explain our results is that a specific histone-DNA

complex protects discrete lengths of DNA from nuclease digestion; thus the fundamental architecture of chromosomes may well be the same in both plants and animals. Since, however, several of the histone classes do show differences between different organisms¹, and yet, at least at the level of this study, their combined effect on DNA is quite similar, comparative studies (potentially leading to inter-species reconstitution experiments) seem an attractive approach to the investigation of the detailed structure of the chromatin particles, and the forces involved in its maintenance. As a recent example of such a comparative study, nuclease digestion of yeast chromatin (which lacks certain classes of histones) was found to give a protected DNA fragment which was both smaller and more homogeneous than that obtained from calf thymus chromatin¹². A number of questions are suggested. When did this subunit structure arise in evolution? What purpose does this chromatin particle serve that requires such a specific length? If it represents simply a structural means to package large amounts of DNA, why is it so unusual that it has been stringently conserved throughout the course of evolution?

This work was supported by a grant from the US Public Health Service to P. H. von Hippel, whom we thank for support and encouragement. We also thank R. Dam, K. Engel, B. Hicks and H. Howard.

JAMES D. MCGHEE
JAMES D. ENGEL

*Institute of Molecular Biology and
Departments of Biology and Chemistry,
University of Oregon,
Eugene, Oregon 97403*

Received December 20, 1974; revised February 14, 1975.

- ¹ DeLange, R. J., and Smith, E. L., *Ann. Rev. Biochem.*, **40**, 279-314 (1971).
- ² DeLange, R. J., Fambrough, D. M., Smith, E. L., and Bonner, J., *J. biol. Chem.*, **244**, 5669-5976 (1969).
- ³ Patthy, L., Smith, E. L., and Johnson, J., *J. biol. Chem.*, **248**, 6834-6836 (1974).
- ⁴ Olins, A. L., and Olins, D. E., *Science*, **183**, 330-332 (1974).
- ⁵ Van Holde, K. E., Sahasrabudhe, C. G., Shaw, B. R., van Bruggen, E. F. J., and Arnberg, A. C., *Biochem. biophys. Res. Commun.*, **60**, 1365-1370 (1974).
- ⁶ Noll, M., *Nature*, **251**, 249-251 (1974).
- ⁷ Clark, R. J., and Felsenfeld, G., *Nature new Biol.*, **229**, 101-106 (1971); *Biochemistry*, **13**, 3622-3628 (1974).
- ⁸ Hewish, D. R., and Burgoyne, L. A., *Biochem. biophys. Res. Commun.*, **52**, 504-510 (1973).
- ⁹ Bonner, J., et al., *Methods in enzymology*, **178**, 3-64 (Academic, New York, 1968).
- ¹⁰ Studier, F. W., *J. molec. Biol.*, **11**, 373-390 (1965).
- ¹¹ Record, M. T., Woodbury, C. P., and Inman, R. B., *Biopolymers*, (in the press).
- ¹² Lohr, D., and Van Holde, K. E., *Science* (in the press).
- ¹³ Sharp, P. A., Sugden, B., Sambrook, J., *Biochemistry*, **12**, 3055-3063 (1973).

Age-dependent excision repair of damaged thymine from γ -irradiated DNA by isolated nuclei from human fibroblasts

THE lifespan of diploid human fibroblasts in culture is limited¹, although there are substantial variations in lifespan with different culturing conditions and growth media²⁻⁴. Before death, the cells pass through a period of senescence, characterised by numerous structural changes⁵. According to the error catastrophe hypothesis, a breakdown of the fidelity of the expression of genetic information resulting in the production of faulty proteins⁶⁻¹¹, and the accumulation of mutations in the genome¹², may represent important mechanisms of cellular ageing. It would be predicted that the deterioration of the quality of the genome would be accelerated by deficient or error-prone DNA repair. Several steps in the excision repair of damaged DNA residues have been demonstrated in mammalian cells exposed to exogenous physical or chemical agents. In the case of ionising radiation, the removal from the DNA of thymine damaged by γ rays was observed in Chinese hamster ovary¹³ and human WI-38 cells¹⁴, and the filling of the resulting gaps, indicated by repair replication or unscheduled synthesis, has been demonstrated in numerous cell lines¹⁵. Recently published experiments¹⁶ have demonstrated that the capacity of skin fibroblasts to perform ultraviolet-induced repair synthesis

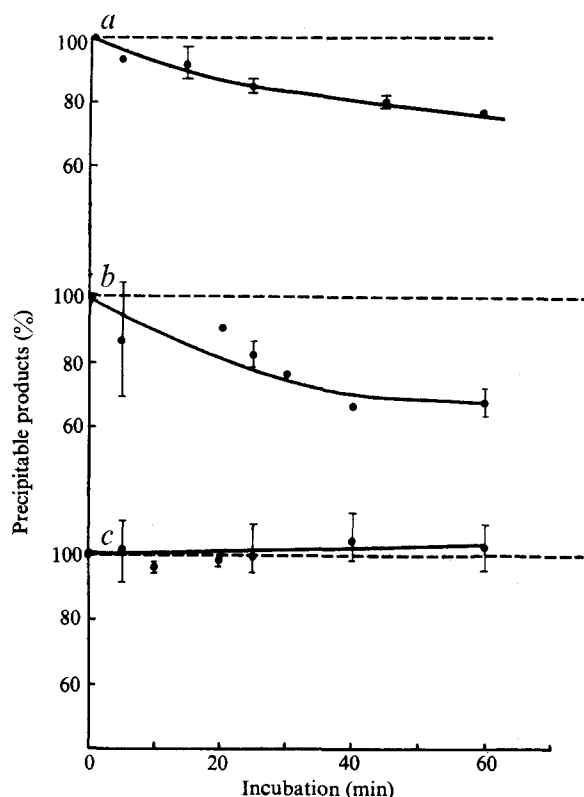


Fig. 1 Excision of thymine radiation products of the t' type from γ -irradiated PM2 DNA by isolated nuclei from (a) young (passage 23), (b) medium-aged (passages 37 and 39) and (c) old cells (passages 43, 47 and 48). Diploid human lung fibroblasts WI-38 were grown in tissue culture according to the method of Hayflick and Moorhead¹. Isolated nuclei were prepared by a modification of the procedure of Berkowitz *et al.* (ref. 21 and J. F. Remsen and P. A. C., unpublished). Cells were collected by trypsinisation into sterile screw-cap bottles (50 ml), washed twice with ice-cold Hanks' balanced salt solution, and centrifuged at 1,200 r.p.m. for 10 min. Homogenisation was then effected in

hypertonic sucrose buffer (0.32 mM sucrose, 0.3% Triton X-100, 1 mM potassium phosphate buffer (pH 7.1), 1 mM Mg^{2+} , 1 mM dithiothreitol) with a Dounce homogeniser, after which the suspensions were centrifuged at 1,200 r.p.m. for 10 min. The homogenisation procedure was repeated and, following centrifugation, the nuclei were washed twice in isotonic sucrose buffer (0.25 M sucrose, 1 mM potassium phosphate buffer (pH 7.1), 1 mM dithiothreitol, 3 mM Ca^{2+}) and the nuclear suspensions centrifuged. In some experiments, nuclei were disrupted by sonication (two treatments for 10 s at the lowest setting) and the completeness of sonication checked using phase contrast microscopy. Pseudomonas phage PM2 DNA labelled with 3H -methyl-thymidine was prepared according to Franklin *et al.*²², and purified on hydroxyapatite before each experiment. This was used as DNA substrate for the experiments using γ -irradiation because it could be prepared essentially free of strand breaks. Irradiation was carried out under non-protective conditions in aerated phosphate buffer with a ^{137}Cs -source; doses used (usually 15 to 25 krad) resulted in the formation of 0.3–0.6% t' , as determined in the acid-precipitable material by the alkaline-acid degradation assay of Hariharan and Cerutti²³. The DNA was passed through a small Sephadex G75 column following irradiation to remove a small amount of low molecular weight material. Although t' represents a major class of radiation products, γ -irradiated DNA contains, in addition, a variety of products derived from the other nucleic acid bases, apyrimidinic²⁴ and, possibly apurinic sites, as well as radiation-induced strand breaks. A second substrate containing primarily 5,6-dihydroxy-dihydrothymine and a small number of apyrimidinic sites (B. E. Dunlap and P. A. C., unpublished) was therefore prepared by the oxidation of polyd(A-T)- 3H -methylthymine with OsO_4 . The conditions of Beer *et al.*²⁵ were used and the oxidation was allowed to continue until the polymer contained 0.3–0.7% t' , or 2–4.5% ring-damaged thymine²⁶. The samples for the excision experiments contained (in a total volume of 150 μ l) 4×10^6 nuclei or sonicated nuclear equivalents, labelled (0.5– 1.5×10^5 c.p.m.) irradiated PM2 DNA or OsO_4 -oxidised polyd(A-T) corresponding to $1-3 \times 10^{-6}$ μ mol t' , 0.01 M phosphate buffer (pH 7.6), 0.09 M NaCl, 1 mM dithiothreitol, 15 U pyruvate kinase, 15 mg ml $^{-1}$ BSA (Fraction V), 2 mM PEP, 0.4 mM ATP, and 0.2 mM of each of the four deoxynucleoside triphosphates. Incubation with substrates was carried out for up to 60 min at 37 °C and the reactions terminated by the addition of 1.9 ml 7% trichloroacetic acid. Thymine radiation products (t') were determined using the above method of Hariharan and Cerutti²³. The ratio of t' in the acid-precipitable material over the total acid precipitable input counts (t'/T_{ppt}) was computed for each time point and expressed as the percentage of the corresponding value for a non-incubated control sample.

increases as a function of the lifespan of the donor species. We report that isolated nuclei or nuclear sonicates from senescent diploid human lung fibroblasts WI-38 have lost their ability to excise from DNA thymine damaged by γ rays.

We have studied the capacity of isolated nuclei and nuclear sonicates from young, medium-aged and old WI-38 cells to excise damaged thymine residues of the 5,6-dihydroxy-dihydrothymine type (t') from exogenous γ -irradiated or osmium tetroxide (OsO_4) oxidised DNA substrates. Our experimental design allows the use of non-irradiated and unlabelled nuclei. Comparison of results obtained with these two substrates allows an assessment of the effects of damage other than t' , in particular, radiation-induced strand breaks, on the repair process (see legend to Fig. 1). The loss of t' from the acid-precipitable exogenous DNA substrate and the amount of undamaged thymine rendered acid-soluble were determined as a function of incubation time.

WI-38 cells of passages 14 and 29 (L. Hayflick, Stanford) were used as starting cultures for the production of cells at the three age groups investigated. Under our culturing conditions, cell death usually occurs at passages 49–52. The age in culture of the WI-38 cells from which the nuclei were prepared is reflected in the passage number (that is, the number of 1:2 subcultures) and was further characterised by the percentage of nuclei which were labelled in a 48 h pulse with 3H -methyl-thymidine according to the method of Cristofalo and Sharf¹⁷. These authors found a decrease with passage number in the percentage of nuclei actively synthesising DNA within a 48 h period, starting approximately at passage 30, and suggested that such data could be used for the character-

isation of the functional state of WI-38 cells. Their results and ours are in agreement. In the following, we refer to cells at passages 15–25 as 'young', to cells at passages 26–42 as 'medium aged', and to cells at passages above 42 as 'old'.

Figure 1a shows that young nuclei of passage 23 and medium-aged nuclei of passages 37 and 39 removed approximately one-quarter to one-third of t' from the γ -irradiated acid-precipitable PM2 DNA within 60 min of incubation at 37 °C; however, no excision of t' was accomplished by old nuclei of passages 43, 47 and 48. Only 2–6% of total thymine label was rendered acid-soluble during 60 min incubation. Excision of t' by nuclei from young and medium-aged cells remained incomplete. The percentage excision within 60 min of incubation is comparable to that accomplished by human skin fibroblasts at similar concentrations of t' (J. F. Remsen, P. V. Hariharan and P. A. C., unpublished). Analogous data (not shown) were obtained for the excision of t' from γ -irradiated PM2 DNA after incubation with nuclear sonicates. The capacity of WI-38 nuclei to excise t' from OsO_4 -oxidised polyd(A-T) also depended on cell age: WI-38 nuclei obtained from young cells at passages 21 and 24 selectively excised 28% of t' within 60 min of incubation at 37 °C; nuclei from medium-aged cells at passage 34 excised 22% of t' ; no excision was observed with old nuclei of passage 47. The excision of t' from OsO_4 -oxidised polyd(A-T) by young and medium-aged nuclei occurs with very high selectivity, the acid solubilisation of thymine label within 60 min of incubation being only 0.6–1%.

The following conclusions can be drawn from our results: Young and medium-aged nuclei up to a passage number of

approximately 42 (corresponding to a percentage labelled nuclei value of 60–70%)¹⁷ possess the capacity for the selective excision of γ -ray damaged or OsO_4 -oxidised thymine (t') from exogenous DNA. The fact that excision occurs from OsO_4 -oxidised polyd(A–T) indicates that neither damage to bases other than thymine nor, in particular, radiation-induced strand breakage is required for removal of t' from DNA. The extent of polymer degradation which accompanies excision is small, particularly for OsO_4 -oxidised polyd(A–T). In this case, only 1.6 undamaged nucleotides are released per ring-damaged thymine within 60 min of incubation. Old nuclei do not accomplish excision of t' either from γ -irradiated PM2 DNA or OsO_4 -oxidised polyd(A–T). The loss of the excision capacity in old nuclei and nuclear sonicates occurs within a few generations (passages 42 to 45)—at least four to five generations before cell death occurs—and seems to occur more precipitously than the loss of the capacity for DNA synthesis, as measured by the percentage labelled nuclei method of Cristofalo and Sharf¹⁷. The loss of repair enzymes during the preparation of nuclei from old but not from young or medium-aged cells could also explain our findings.

The observed loss of the excision repair capacity for γ -ray-induced thymine damage by nuclei from senescent WI-38 cells does not allow conclusions concerning the validity of error catastrophe hypotheses of cellular ageing^{10–12}. Because the cells were found to survive for several generations after the repair deficiency had first manifested itself, however, the loss of this function does not seem to be a direct consequence of cell death. It is, on the other hand, impossible, on the basis of our data, to decide whether the observed repair deficiency is one of the causes or a consequence of senescence in cultured WI-38 cells. Painter and collaborators^{18,19}, found a decline in the ability of WI-38 cells to undergo ultraviolet or γ -ray-induced repair replication only in the last one or two passages before cell death occurred. Hart and Setlow²⁰ observed a concomitant decrease in the capacity for ultraviolet-induced unscheduled synthesis and scheduled DNA synthesis in senescent WI-38 cells. We have, however, investigated an earlier step in γ -ray excision repair than in those studies measuring unscheduled synthesis or repair replication.

This work was supported by a grant from the NIH and a contract from the US Atomic Energy Commission.

M. R. MATTERN
P. A. CERUTTI

Department of Biochemistry,
University of Florida,
Gainesville, Florida 32610

Received May 28; revised December 17, 1974.

- ¹ Hayflick, L., Moorehead, P. S., *Expl. Cell. Res.*, **25**, 585–621 (1961).
- ² Packer, L., and Smith, J. R., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4763–4767 (1974).
- ³ Macieira-Coelho, A., *Experientia*, **22**, 390–391 (1966).
- ⁴ Cristofalo, V. J., in *Ageing in Cell and Tissue Culture* (edit. by Holeckova, E., and Cristofalo, V. J.), (Plenum, New York, 1970).
- ⁵ Lipetz, J., and Cristofalo, V. J., *J. Ultrastruct. Res.*, **39**, 43–55 (1972).
- ⁶ Wang, K.-M., Rose, N. R., Bartholomew, E. A., Balzer, M., Berde, K., and Foldvary, M., *Expl. Cell. Res.*, **61**, 357–364 (1970).
- ⁷ Cristofalo, V. J., Parris, N., and Kritchevsky, D., *J. Cell Physiol.*, **69**, 263–271 (1967).
- ⁸ Holliday, R., and Tarrant, G. M., *Nature*, **238**, 26–30 (1972).
- ⁹ Holliday, R., *Nature*, **248**, 762–763 (1974).
- ¹⁰ Orgel, L. E., *Proc. natn. Acad. Sci. U.S.A.*, **49**, 517–521 (1963).
- ¹¹ Orgel, L. E., *Nature*, **244**, 441–445 (1973).
- ¹² Curtis, H. J., *Adv. Genet.*, **16**, 305–324 (1971).
- ¹³ Mattern, M. R., Hariharan, P. V., Dunlap, B. E., and Cerutti, P. A., *Nature new Biol.*, **245**, 230–232 (1973).
- ¹⁴ Cerutti, P. A., *Naturwissenschaften*, **61**, 51–59 (1974).
- ¹⁵ Painter, R. B., *Current Topics in Radiation Research*, **7**, 45–70 (1970).
- ¹⁶ Hart, R. W., and Setlow, R. B., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2169–2173 (1974).
- ¹⁷ Cristofalo, V. J., and Sharf, B., *Expl. Cell. Res.*, **76**, 419–427 (1973).
- ¹⁸ Painter, R. B., Clarkson, J. M., and Young, B. R., *Rad. Res.*, **56**, 560–564 (1973).
- ¹⁹ Clarkson, J. M., and Painter, R. B., *Mut. Res.*, **23**, 107–112 (1974).
- ²⁰ Hart, R., and Setlow, R. B., in *Fifth Int. Congr. Rad. Res.*, Seattle (1974).
- ²¹ Berkowitz, D. M., Kakefuda, T., and Sporn, M. B., *J. Cell. Biol.*, **42**, 851–855 (1969).
- ²² Franklin, R., Salditt, M., and Silbert, P. A., *Virology*, **38**, 627–640 (1968).
- ²³ Hariharan, P. V., and Cerutti, P. A., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3532–3536 (1974).
- ²⁴ Dunlap, B. E., and Cerutti, P. A., *FEBS Lett.* (in the press).
- ²⁵ Beer, M., Stern, S., Carmalt, D., and Mohlenrich, K., *Biochemistry*, **5**, 2283–2288 (1966).

Determination of recognition sites of T4 RNA ligase on the 3'-OH and 5'-P termini of polyribonucleotide chains

RNA LIGASE which was discovered by Silber *et al.*¹ in T4-infected *Escherichia coli* cells, catalyses an ATP-dependent, intramolecular joining reaction of 5'-P and 3'-OH termini of various homopolyribonucleotides^{1,2}. The shortest circularising polyadenylate was shown to be (pA)₈, the optimal chain length for the reaction being 10–30 (ref. 2). These facts suggest that the substrate for T4 RNA ligase is a pair of suitably juxtaposed, 3'-OH and 5'-P termini, each a few single stranded residues long².

An intermolecular joining RNA ligase reaction can be induced by suitably juxtaposing termini of different polyribonucleotide chains. Thus a pair of tRNA^{Phe} half-molecules, fragments 1–36 and 38–74, could be joined by RNA ligase at the termini of the severed anticodon loop³. To effect intermolecular joining reactions of non-complexed polyribonucleotide chains, however, other means must be sought to juxtapose their termini, such as increasing the polyribonucleotide concentration (O. C. Uhlenbeck, personal communication). We have therefore investigated the minimal recognition sites on the 3'-OH and 5'-P termini of RNA ligase substrates. We have tested the ability of short oligonucleotides varying in chain length and carrying only one functional terminus to participate in RNA ligase-catalysed reactions. As oligonucleotides carrying only a 3'-OH function, we generally used (Ap)_nA where n ≥ 4, while

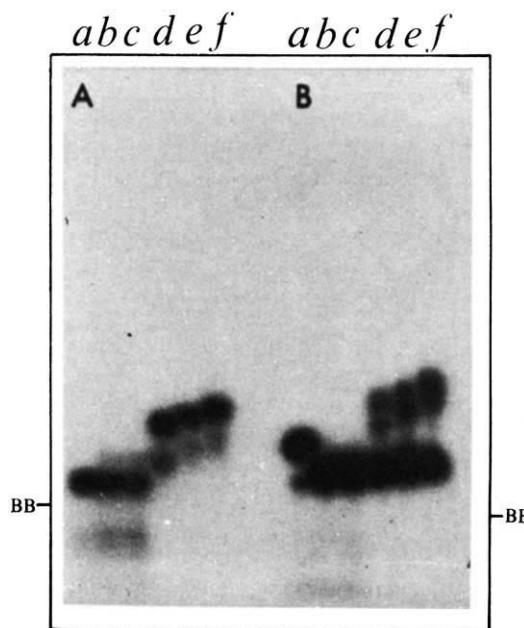


Fig. 1 A, Joining of (Ap)_nA with 5' ³²P (pA)₇. 5' ³²P-labelled oligoadenylates, (Ap)_nAs and RNA ligase were prepared as before². The reaction mixtures (0.01 ml) contained: 5' ³²P (pA)₇ (2,000 c.p.m. pmol⁻¹) 0.65 μM, Tris-HCl buffer pH 7.5, 50 mM; MgCl₂ 10 mM, dithiothreitol 1 mM, ATP 0.5 mM, (Ap)_nA as indicated 2 mM, and 0.05 U of RNA ligase. The reaction mixture was incubated at 37 °C for 3 h. An aliquot of 5 μl was then mixed with 5 μl of 50% sucrose, 0.1 M Tris-borate, pH 8.3, 0.01 M EDTA, 0.01% bromophenol blue, applied to a 20% polyacrylamide 7 M urea gel slab and separated by electrophoresis as previously described². a, (pA)₇; b, reaction mixture with no (Ap)_nA added; c, (Ap)₄A added; d, (Ap)₂A added; e, (Ap)₃A added; f, (Ap)₁A added; BB, bromophenol blue R_{BB} values of (Ap)_nAs were obtained by separating a mixture of (Ap)_nA (n = 4–17) on the same gel. The bands were detected by staining with 'Stains-all'¹⁷ pure (Ap)₄A served as a reference band. B, Joining of (Ap)_nA with 5' ³²P (pA)₁₀. The reaction mixtures were essentially as described in (A) except that (pA)₇ was substituted by 1.5 μM 5' ³²P (pA)₁₀.

periodate-treated $(pA)_n$ oligonucleotides ($n = 2, 3, 4$) served for study of the substrate requirements at the 5'-P terminus.

Two kinds of assay were carried out: first, linear joining of either kind of the short monofunctional oligonucleotide to one of the termini of a 5'- ^{32}P $(pA)_7$ and second, their competition with $(pA)_{10}$ circularisation. The shortest circularising polyadenylate was previously shown to be $(pA)_8$ (ref. 2). A hepta-adenylate will not circularise. In the presence of RNA ligase and excess of short dephosphorylated oligo As, however, $(pA)_7$ participated in a linear joining reaction. When samples of these reaction mixtures were analysed by polyacrylamide-urea gel electrophoresis, it was found that, when reacted, $(pA)_7$ was converted into a longer oligonucleotide migrating like a corresponding $(Ap)_7 + nA$ marker (Fig. 1A). This oligonucleotide was therefore the linear joining product of $(Ap)_nA$ with $(pA)_7$. $ApApA$ was the shortest oligonucleotide to support such a reaction while ApA had no effect. High concentrations of $(Ap)_nA$ were required to elicit linear joining (Fig. 2) at rates

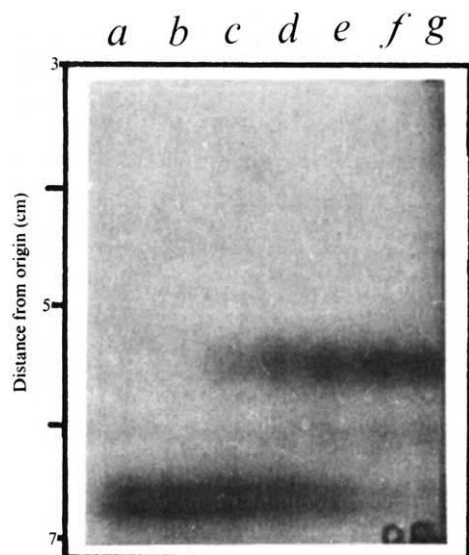


Fig. 2 Effect of $(Ap)_3A$ concentration on its joining to $(pA)_7$. The reaction mixtures (0.04 ml) contained 5'- ^{32}P $(pA)_7$, 0.5 μM , Tris HCl, pH 7.5, 50 mM; $MgCl_2$ 10 mM, dithiothreitol 1 mM, ATP 0.5 mM, $(Ap)_3A$ as indicated and 0.5 U of RNA ligase. The mixture was incubated at 37 °C; after 2 h 5 μl aliquots were separated by electrophoresis as described for Fig. 1A. *a*, $(pA)_7$; *b*, reaction mixture without $(Ap)_3A$; *c*, 0.075 mM $(Ap)_3A$; *d*, 0.15 mM $(Ap)_3A$; *e*, 0.3 mM $(Ap)_3A$; *f*, 0.6 mM $(Ap)_3A$; *g*, 1.2 mM $(Ap)_3A$.

comparable with those seen in the circularisation of oligoadenylates. In standard assay conditions², the apparent K_m of $(Ap)_3A$ (at $5.10^{-7} M$ $(pA)_7$) was about $8.10^{-4} M$, about 1,000 times higher than the K_m of a well-circularising substrate² with a pair of 3'-OH and 5'-P termini.

Since short, dephosphorylated oligoadenylates were shown to serve as 3'-OH acceptor termini in an RNA ligase reaction, we expected them also to compete with the 3'-OH of a circularising oligoadenylate, such as $(pA)_{10}$, for the 5'-P terminus. As a result, linear rather than circular joining products would be formed. Figure 1B shows the analysis, by gel electrophoresis, of reaction mixtures containing RNA ligase, 5'- ^{32}P $(pA)_{10}$ and a short dephosphorylated oligoadenylate in about 500-fold excess over $(pA)_{10}$. In the mixture containing enzyme and $(pA)_{10}$ only, complete circularisation took place as indicated by the conversion of linear $(pA)_{10}$ ($R_{BB} = 0.85$) into a faster migrating product ($R_{BB} = 0.90$), as previously described². Addition of ApA had no effect, while $(Ap)_2A$, $(Ap)_3A$ and $(Ap)_4A$ induced the formation, in addition to circular $(pA)_{10}$, of two new, more slowly migrating products. Of these, the slower band comigrated with $(Ap)_{10} + nA$ marker, and probably resulted from linear joining of $(Ap)_nA$ to $(pA)_{10}$ in competition

with the circularisation reaction. The yield of the linear joining product of $(pA)_{10}$ and $(Ap)_nA$ comprised about 10% of the original labelled substrate. The slower new product resulted from the linear addition of $(Ap)_nA$ to 5'- ^{32}P $(pA)_8p$ contaminant, present in the $(pA)_{10}$ preparation (see the faster band in slot *a*, Fig. 1B). This contaminating oligoadenylate cannot circularise and is completely converted into a linear joining product in presence of $(Ap)_nAs$.

To study the requirements of RNA ligase at the 5' terminus of its substrate we tested the ability of short, 5' phosphorylated, 3' periodate oxidised, oligoadenylates $((pA)_n)^{ox}$ in joining to 5'- ^{32}P $(pA)_7$ and in competition with 5'- ^{32}P $(pA)_{10}$ circularisation. Figure 3A and B shows the analysis of products of such reactions by polyacrylamide gel electrophoresis. All the $((pA)_n)^{ox}$ oligoadenylates tested, starting from a dinucleotide, participated in linear joining to $(pA)_7$ and to $(pA)_{10}$, in the latter case at the expense of circularisation. Again, relatively high concentrations of $((pA)_n)^{ox}$ were required to elicit detectable linear joining reactions.

The experiments with dephosphorylated oligoadenylates (Fig. 1A and B) indicated that the RNA ligase recognition site on the 3'-OH terminus consists of no more than a trinucleoside diphosphate ($ApApA$) stretch. A dinucleoside monophosphate (ApA) is not sufficient. However, a dinucleoside diphosphate cannot be ruled out as a minimal 3'-OH recognition site. Since the experiments with 5'- $((pA)_n)^{ox}$ indicated that $(pA)_2$ and possibly smaller portions of the 5'-P terminus are recognised by RNA ligase, one might expect that $(pA)_2$ would be polymerised by RNA ligase, were it recognised also as a 3'-OH terminus. Our preliminary results (unpublished) confirm this expectation.

The minimal 5'-P recognition site is possibly less than a dinucleoside diphosphate (Fig. 3). It was previously found in the RNA ligase-catalysed loop closure reaction of yeast tRNA^{Phe} fragments 1-36 and 38-74 (ref. 3), that no more than one single stranded nucleotide residue (emanating from a double helix) is required for recognition of the 5'-P terminus. Whether the 5'-P recognition site extends beyond the first nucleotide residue to include some portion of the adjacent residue is unknown. The possibility that T4 RNA ligase can join nicked, double stranded

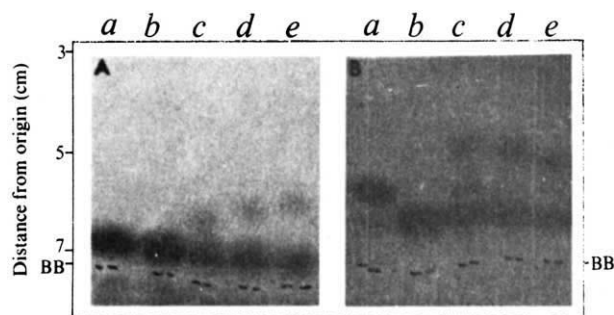


Fig. 3 A, Joining of $((pA)_n)^{ox}$ to 5'- ^{32}P $(pA)_7$. $((pA)_2)^{ox}$, $((pA)_3)^{ox}$ and $((pA)_4)^{ox}$ were prepared by controlled digestion of poly(A) by sheep kidney nuclease⁶ followed by fractionation of the digest on a DEAE-cellulose column. To oxidise their 3' ribose these oligonucleotides were treated with sodium periodate as follows: the reaction mixture (0.025 ml) contained 10 mM oligonucleotide and 15 mM sodium periodate and was incubated for 30 min at 0 °C, at the end of which 1 μl of 0.25 M ethylene glycol was added. The solution of the periodate-treated oligonucleotide was used as such in the following experiment. The joining reaction mixture (0.02 ml) contained $4 \times 10^{-7} M$ $(pA)_7$, Tris-HCl, pH 7.5, 50 mM, $MgCl_2$ 10 mM, dithiothreitol 1 mM, ATP 0.5 mM, $((pA)_n)^{ox}$ as indicated and 0.1 U RNA ligase. After 2 h of incubation at 37 °C, 3 μl samples were separated by electrophoresis as described in legend to Fig. 1A. 1, $(pA)_7$; 2, reaction mixture without $((pA)_n)^{ox}$; 3, 1.2 mmol of $((pA)_2)^{ox}$ added; 4, 1.2 mmol of $((pA)_3)^{ox}$ added; 5, 0.8 mmol of $((pA)_4)^{ox}$ added. B, Joining of $((pA)_n)^{ox}$ to 5'- ^{32}P $(pA)_{10}$. The reaction mixture was similar to that in A, except for containing $1.8 \times 10^{-6} M$ of $(pA)_{10}$ instead of $(pA)_7$. *a*, $(pA)_{10}$; *b*, reaction mixture without $((pA)_n)^{ox}$; *c*, 0.8 mM of $((pA)_2)^{ox}$ added; *d*, 1.2 mM of $((pA)_3)^{ox}$ added; *e*, 1.2 mM of $((pA)_4)^{ox}$ added.

RNA^{1,4} must also be considered; a definitive answer awaits studies with carefully defined substrates.

Our results and those of Uhlenbeck (personal communication) show that T4 RNA ligase can catalyse the joining reaction between unassociated polyribonucleotide chains. In view of the high substrate concentrations required to support such reactions, we consider it unlikely that selective joining reactions of non-complexed RNA molecules are catalysed by the enzyme *in vivo*. The high affinity of RNA ligase for its substrates in the loop closure³ and circularisation reactions, as well as the competition experiments between intra- and intermolecular joining indicate that a pair of suitably apposed 3'-OH and 5'-P termini of RNA molecules constitutes the *in vivo* substrate of RNA ligase. The requirement that the pair of termini be suitably apposed can be met when (1) they belong to different chains that are partially hybridised, as in severed loops, or (2) when proximity is mediated by a specific factor, or (3) when the termini belong to one chain capable of circularisation. A possibility that cannot be dismissed is that this T4-induced enzyme evolved as a host RNA-modifying function. Other possible participants of such a pathway could be two other early induced T4 enzymes—polynucleotide kinase⁵ and the nuclease that specifically cleaves one of the isoaccepting host tRNA^{Lys} species⁶.

The linear joining reaction catalysed by RNA ligase offers important new synthetic possibilities. It permits joining of tri- and higher oligoribonucleotides in a stepwise manner to a 5'-P terminus of a growing chain. The following scheme is suggested for one step in such a synthesis. First, a dephosphorylated oligonucleotide is joined in an RNA ligase-catalysed reaction to a 5'-P initiator polynucleotide, the 3'-OH terminus of which is blocked. Second, the 5'-OH terminus of the product is phosphorylated by polynucleotide kinase, ready for a subsequent joining step. The synthesis may be programmed in such a way as to generate single stranded chains only; these in turn may be hybridised and subsequently joined in an RNA ligase-catalysed loop closure reaction³.

This work was supported by grants from the United States-Israel binational Science Foundation and by the US National Science Foundation.

GABRIEL KAUFMANN*
NEVILLE R. KALLENBACH

Department of Biology,
University of Pennsylvania,
Philadelphia, Pennsylvania 19174

Received October 2, 1974.

*On leave from the Biochemistry Department, Weizmann Institute of Science, Rehovot, Israel; Present address: Biology Department, Massachusetts Institute of Technology, Cambridge, Massachusetts.

- ¹ Silber, R., Malathi, V. G., and Hurwitz, J., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3009-3013 (1972).
- ² Kaufmann, G., Klein, T., and Littauer, U. Z. *FEBS Lett.* (in the press).
- ³ Kaufmann, G., and Littauer, U. Z., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 000 (1974).
- ⁴ Linné, T., Öberg, B., and Phillipson, L., *Eur. J. Biochem.*, **42**, 157-167 (1974).
- ⁵ Richardson, C. C., *Proc. natn. Acad. Sci. U.S.A.*, **54**, 158-165 (1965).
- ⁶ Yudelevich, A. C., *J. molec. Biol.*, **60**, 21-29 (1971).
- ⁷ Dahlberg, A. E., Dingman, C. W., and Peacock, A. C., *J. molec. Biol.*, **41**, 139 (1971).
- ⁸ Kasai, K., and Grunberg-Manago, M., *Eur. J. Biochem.*, **1**, 152-167 (1967).

Sequential translation of capsid and membrane protein genes of alphaviruses

INVESTIGATIONS of the multiplication of alphaviruses such as Semliki Forest virus (SFV) or Sindbis virus have suggested that the three glycoproteins¹ (E1, E2 and E3) located in the viral envelope are initially synthesised as a large precursor polypeptide which contains the amino acid sequences of them all²⁻⁷. This protein, NVP97 (ref. 5), is cleaved to give E1 and another precursor polypeptide, NVP63, which is in turn cleaved to give E2 and E3 (refs 6 and 7). By analogy with the picornavirus system, it has been widely suggested that all the viral structural proteins are derived by cleavage of a common

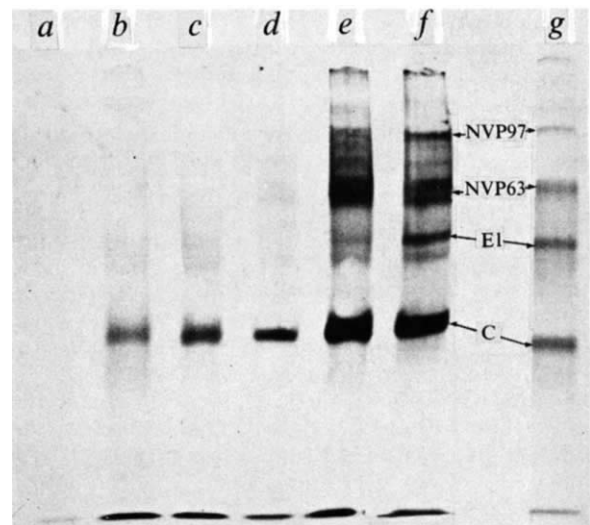


Fig. 1 Monolayer cultures of BHK cells in glass scintillation vials (about 10^6 cells per vial) infected 5.5 h previously with SFV (20 PFU per cell) were exposed to minimal medium (Earle's salts containing 2% dialysed calf serum) to which 225 mM NaCl (ref. 14) had been added. After 40 min the hypertonic medium was replaced with isotonic minimal medium containing ³⁵S-methionine (200 Ci mmol⁻¹; 10 μ Ci ml⁻¹). Actinomycin D (1 μ g ml⁻¹) was present at all times after infection of the cells. As Saborio *et al.*¹⁴ found, exposure of the cells to the salt-containing medium resulted in cessation of protein synthesis within 15 min. After restoration of isotonicity protein synthesis resumed without delay at a rate nearly equal to that before shutoff. After (a) 1 min, (b) 2 min, (c) 3 min, (d) 4 min, (e) 5 min or (f) 6 min, incorporation was stopped by freezing the cells in dry ice-methanol mixture¹¹ and adding 5% TCA containing 1% casamino acids. After thawing the cell sheet, washing twice with 5% TCA, and twice with ethanol-ether (1:3 v/v), it was dissolved in 2% SDS, 1% 2-mercaptoethanol, 0.1 M Tris-HCl, pH 9.0. The proteins were alkylated and analysed on 10% polyacrylamide gel slabs, which were dried and autoradiographed⁸. Proteins labelled for 2 min and chased for 12 min in similar cultures of untreated cells (g) are shown for comparison. Virus-specific proteins are identified using the nomenclature of Morser *et al.*⁵.

precursor and, indeed, proteins of a size sufficient to encompass the viral capsid protein, as well as the envelope proteins, have been found in infected cells treated with amino acid analogues and inhibitors of proteolytic enzymes^{10,11}, and in cells infected with certain classes of temperature-sensitive mutant^{2,3,12,13}. Doubts have been cast on the validity of the analogy, however, by reports^{8,9,16-19} that cell-free translation of SFV or Sindbis virus mRNA in several systems resulted in the production of discrete viral proteins. This situation is in marked contrast to that of translation of picornavirus RNA, in which a spectrum of polypeptides of various lengths is formed by premature termination at many points along the messenger; since no post-translational cleavage seems to take place, no polypeptides found in the mature virus or in infected cells are formed^{20,21}. The possibility arose, therefore, that the alphavirus capsid protein and envelope proteins are synthesised independently.

Saborio *et al.*¹⁴ have shown that restoring to isotonicity cultures of HeLa cells infected with polio virus, which had been treated with medium containing an elevated concentration of NaCl, induced the synchronous initiation of protein synthesis and allowed the order in which the viral proteins were synthesised to be examined. Here, I report similar experiments with SFV-infected BHK cells, which clearly demonstrate the processes of sequential synthesis and cleavage taking place during the formation of all the alphavirus structural proteins.

Autoradiographs of SDS-polyacrylamide gel slabs containing the proteins synthesised during a pulse experiment are shown in Fig. 1. By comparison with the proteins synthesised in non-synchronised cells, capsid protein is first apparent 2 min after restoring isotonicity, whereas radioactivity does not become associated with discrete envelope proteins or their

precursors until after 5–6 min, when NVP97, NVP63, and an envelope protein (whose identity is discussed below) all emerge together from the background radioactivity resulting from nascent chains and residual host protein synthesis. If the pulse of radioactivity is followed by a chase period a different picture emerges (Fig. 2). After a 1 min pulse only radioactivity which could be chased into capsid protein had been incorporated. As the length of the pulse increases there is a sequential appearance of envelope-specific material; NVP97 and NVP63 are present after a 2 min pulse and a protein migrating with the envelope proteins of the virus appears after a 4 min pulse. Large proteins of molecular weight greater than 100,000 are also apparent after a 3 min pulse and chase.

The fact that only capsid protein is labelled after a 1 min pulse and chase strongly suggests the existence of a single initiation site at the beginning of the capsid protein cistron for the translation of all the virus structural proteins. If there were two independent initiation sites for the capsid and envelope proteins, products originating from both sites would be expected to be labelled in a pulse-chase experiment, no matter how short the pulse. Other evidence, derived from work on the cell-free translation of 26S RNA, and on the kinetics with which synthesis of different viral proteins is switched off in cells treated with hypertonic medium, is also consistent only with the existence of a single initiation site for the synthesis of all the structural proteins of the virus (J. C. S. C. and S. I. T. Kennedy, unpublished).

From the time of first appearance of discrete envelope protein and precursors in the pulse experiment (Fig. 1), it seems that complete translation of the genes for the structural proteins (total molecular weight about 140,000) takes about 5 min. Assuming a constant rate of ribosome movement, the time required to translate the region corresponding to the capsid protein (molecular weight 36,500) may be estimated to be approximately 1.3 min. The appearance of capsid protein after a 2 min, but not a 1 min, pulse is in agreement with this estimate and indicates that it is cleaved from the growing peptide chain very shortly after translation of envelope-specific material begins. It is clearly not necessary that any precursor protein be completed before this cleavage occurs. The small quantities of high molecular weight proteins which can be seen after a 3 min pulse and chase may represent the precursor which has escaped the capsid protein cleavage mechanism. It is not clear whether these molecules can be processed to give viral

proteins or whether they represent errors which cannot be corrected, but in any event they are certainly not on the main pathway of synthesis of the proteins.

The next proteins to be labelled after the capsid protein in the pulse-chase experiment are the envelope precursors NVP97 and NVP63. The fact that NVP63 is labelled in the absence of radioactivity in the envelope region of the gels indicates that it is NVP63 which is translated immediately after the capsid protein. The absence of labelled envelope protein also means that NVP63 is not cleaved to give observable E2 and E3 during the short chase periods used here. This may mean that NVP63 is an intermediate with a relatively long half life, or that this cleavage is closely connected with the release of virus by budding through the cell membrane, as has been previously suggested^{6,15}; if this were the case, significant quantities of E2 and E3 would not accumulate inside the cell.

Radioactivity finally appears in the envelope region of the gel only after a 4 min pulse in the pulse-chase experiment. Since NVP63 does not seem to be cleaved during the period of this experiment, the labelled envelope protein must be E1. The simultaneous appearance of NVP97, NVP63 and E1 after a 5 min labelling period in the pulse experiment indicates that the cleavage to give NVP63 and E1 takes place only after completion of their precursor (NVP97).

The sequential appearance of the proteins in the pulse-chase experiment combined with the demonstration of a single site for their synthesis indicates that the order of genes for the structural proteins of the virus is C–E2/E3–E1. It is not possible to order E2 and E3 from the present data since the cleavage to give these two products was not detected. It should be possible to arrange them in the correct order by observation of complete virions released during this type of experiment.

These results show that there is a striking parallel between the synthesis of alphavirus structural proteins and that of picornavirus proteins in that, in both cases, initiation occurs at a single site, the capsid proteins are synthesised first and they are cleaved rapidly from the nascent peptide chain. In the alphavirus system this rapid cleavage may be related to the fact that the remainder of the proteins are destined for glycosylation and incorporation into membranes. The removal of the capsid protein would thus avoid its association with membranes and allow the rapid insertion into nucleocapsid particles observed by Söderlund²².

I thank Dr I. Kennedy for discussions, Miss Celia Bruton for technical assistance, and the Cancer Research Campaign for support.

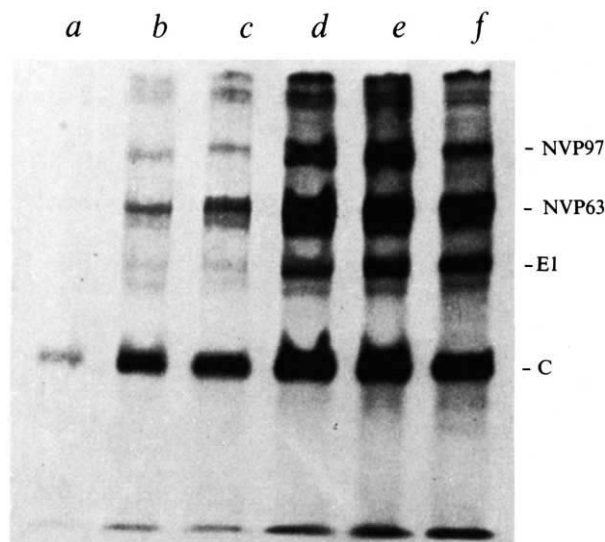
J. C. S. CLEGG

Department of Biological Sciences,
University of Warwick,
Coventry CV4 7AL, UK

Received January 20, 1975.

- 1 Garoff, H., Simons, K., and Renkonen, O., *Virology*, **61**, 493–504 (1974).
- 2 Schlesinger, M. J., and Schlesinger, S., *J. Virol.*, **11**, 1013–1016 (1973).
- 3 Strauss, J. H., Burge, B. W., and Darnell, J. E., *Virology*, **37**, 367–376 (1969).
- 4 Ranki, M., *J. gen. Virol.*, **15**, 59–67 (1972).
- 5 Morser, M. J., Kennedy, S. I. T., and Burke, D. C., *J. gen. Virol.*, **21**, 19–29 (1973).
- 6 Schlesinger, S., and Schlesinger, M. J., *J. Virol.*, **10**, 925–932 (1972).
- 7 Simons, K., Keränen, S., and Kääriäinen, L., *FEBS Lett.*, **29**, 87–91 (1973).
- 8 Clegg, J. C. S., and Kennedy, S. I. T., *FEBS Lett.*, **42**, 327–330 (1974); *Europ. J. Biochem.* (in the press).
- 9 Simmons, D. T., and Strauss, J. H., *J. molec. Biol.*, **86**, 397–409 (1974).
- 10 Pfefferkorn, E., and Boyle, M. K., *J. Virol.*, **9**, 187–188 (1972).
- 11 Morser, M. J., and Burke, D. C., *J. gen. Virol.*, **22**, 395–409 (1974).
- 12 Scheele, C. M., and Pfefferkorn, E., *J. Virol.*, **5**, 329–337 (1970).
- 13 Waite, M. R. F., *J. Virol.*, **11**, 198–206 (1973).
- 14 Saborio, J. L., Pong, S.-S., and Koch, G., *J. molec. Biol.*, **85**, 195–211 (1974).
- 15 Schlesinger, M. J., Schlesinger, S., and Burge, B. W., *Virology*, **47**, 539–541 (1972).
- 16 Cancedda, R., and Schlesinger, M. J., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1843–1847 (1974).
- 17 Cancedda, R., Swanson, R., and Schlesinger, M. J., *J. Virol.*, **14**, 652–663; *ibid.*, 664–671 (1974).
- 18 Wengler, G., Beato, M., and Hackemack, B. A., *Virology*, **61**, 120–128 (1974).
- 19 Smith, A. E., Wheeler, T., Gianville, N., and Kääriäinen, L., *Eur. J. Biochem.*, **49**, 101–110 (1974).
- 20 Kerr, I. M., Brown, R. E., and Tovell, D. R., *J. Virol.*, **10**, 73–81 (1972).
- 21 Öberg, B. F., and Shatkin, A. J., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3589–3593 (1972).
- 22 Söderlund, H., *Intervirology*, **1**, 354–361 (1973).

Fig. 2 Infected cells were treated as in Fig. 1 except that after incorporation of ³⁵S-methionine, the radioactive medium was replaced with minimal medium containing 1 mM non-radioactive methionine (pulse-chase experiment), and further incubated for 15 min after restoring isotonicity, when the cell sheets were frozen and processed as in Fig. 1. Cells were labelled for (a) 1 min, (b) 2 min, (c) 3 min, (d) 4 min, (e) 5 min or (f) 6 min after restoration of isotonicity.



Purification and ultrastructure of Aleutian disease virus of mink

ALEUTIAN disease of mink is an immune complex disease induced by viral infection. Major abnormalities include glomerulonephritis, arteritis, plasmacytosis and hypergammaglobulinaemia^{1,2}. Infectious virus-antibody complexes have been found in the serum of infected mink³, and deposits of viral antigen, antibody and complement have been demonstrated in glomerular and arterial lesions⁴⁻⁶. Detailed study of Aleutian disease virus (ADV) has been hampered by an inability to isolate and purify it by conventional techniques, mainly because ADV obtained from mink tissue is complexed with specific antibody⁷. The immune complexes are not sufficiently homogeneous for purification by physical or chemical properties. Applying techniques that dissociate antigen-antibody complexes to crude preparations of ADV, Cho and Ingram purified viral antigenic material^{8,9}. With the electron microscope they observed virus-like particles in both immune precipitates and viral antigen from CsCl gradients. Although the immune precipitates were infectious for mink, lack of quantitative infectivity data on the CsCl gradient fractions prevented identification of these particles as ADV. We have now succeeded in purifying similar virus particles and have identified them as ADV by quantitative infectivity titration.

The Utah I ADV strain⁴ (obtained from Dr D. Porter in fourth passage as a 10% liver suspension) was propagated and assayed in sapphire mink. Virus stocks were 10% spleen homogenates prepared from pooled spleens of mink inoculated intraperitoneally 9-10 d previously with 0.5 ml of the previous passage virus stock. Ten per cent spleen homogenates were made in phosphate-buffered saline containing 0.002 M ethylene diamine tetraacetate (PBS-EDTA). Homogenates were sonicated

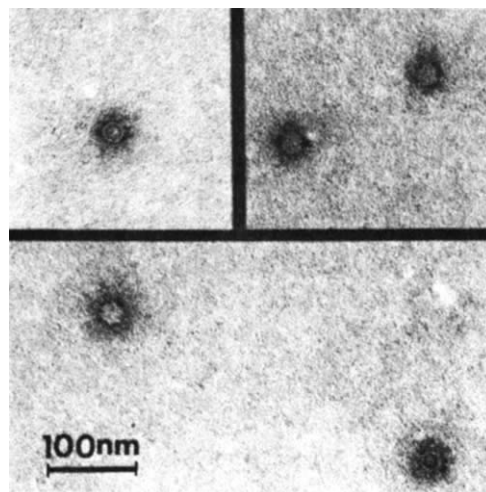


Fig. 2 Electron micrographs of purified ADV pelleted from CsCl gradient fraction 9. Diameter of intact virions is 23 nm. Apparently hollow, ring structured virions are seen in upper left and lower right. Preparations were made with 2% sodium tungstosilicate as negative contrast material.

for 60 s in a Biosonik sonicator (Bronwill Scientific, Rochester), clarified by centrifugation at 500g for 10 min and stored in small volumes at -70°C . We used the seventh passage of Utah I ADV which had a titre in sapphire mink of $2 \times 10^6 \text{ LD}_{50} \text{ ml}^{-1}$ ($2 \times 10^7 \text{ LD}_{50} \text{ g}^{-1}$ of spleen). For further purification 10-ml samples of homogenate were thawed, and debris was removed by centrifugation at 500g for 5 min and at 6,000g for 10 min. The infectious material was pelleted from the clarified suspension by ultracentrifugation at 243,000g for 90 min. Pellets were resuspended in 3 ml of PBS-EDTA with the aid of sonication and shaken with 7 ml of ether at room temperature for 5 min to extract lipids from tissue membrane material. The phases were separated by centrifugation at 500g for 5 min, and the aqueous phase was mixed with an equal volume of 0.1 M glycine-HCl buffer, pH 2.8. The mixture was incubated for 1 h at room temperature to dissociate antibody-virus immune complexes and ultracentrifuged as before to pellet the ADV and leave eluted antibodies in solution. Pellets were resuspended in PBS-EDTA by sonication, mixed with CsCl to a final density of 1.38 g ml^{-1} and ultracentrifuged for 42 h at 83,000g (maximum) at 4°C . Fractions were collected through a hole in the bottom of the tube, and the densities were measured by weighing 0.1-ml samples. Fractions were then dialysed against PBS, tested for viral antigen by counter immunoelectrophoresis (CIEP)⁷, and stored at -70°C . Infectivity was assayed by intraperitoneal inoculation of two to four sapphire mink with 0.5-ml volumes of serial tenfold dilutions of the dialysed fractions. Mink were tested monthly for hypergammaglobulinaemia and specific anti-ADV antibody. Those that remained clinically and serologically normal for 6 months were considered uninfected. Aleutian disease was confirmed at necropsy in all serologically positive mink.

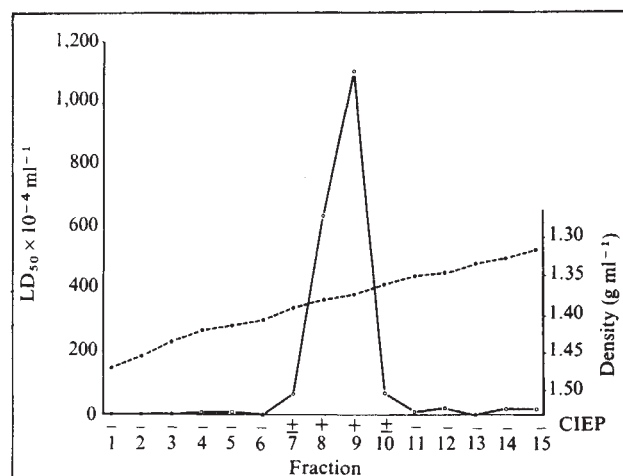


Fig. 1 CsCl gradient purification of ADV. $\circ$, $\text{LD}_{50} \times 10^{-4} \text{ ml}^{-1}$; $\bullet$, density (g ml^{-1}). Results of CIEP tests on individual fractions are shown along the abscissa.

Table 1 ADV infectivity titration of CsCl gradient fractions

		Fraction	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Dilution Inoculated	10^{-4}		0/2*	0/2	0/2	1/2	1/2	0/2	2/2	2/2	2/2	2/2	1/2	2/2	1/2	2/2	2/2
	10^{-5}								2/2	2/2	2/2	2/2	1/2	1/2	0/2	1/2	1/2
	10^{-6}								0/2	2/2	4/4	0/2	0/2	0/2	0/2	0/2	0/2
	10^{-7}										1/4						
$\text{LD}_{50} \times 10^{-4} \text{ per } 0.5 \text{ ml}$			<1	<1	<1	1	1	<1	32	>320	560	32	3.2	10	1	10	10

Each mink inoculated intraperitoneally with 0.5 ml of dilutions indicated. LD_{50} calculated by Spearman-Kärber method. Values plotted as $\text{LD}_{50} \times 10^{-4} \text{ ml}^{-1}$ in Fig. 1.

* Number of mink affected with AD/number of mink inoculated.

A summary of results is shown in Fig. 1, and the detailed data from the infectivity titration are shown in Table 1. Although ADV infectivity was present throughout most of the CsCl gradient, a sharp peak of infectivity was concentrated at a density of 1.37–1.38, corresponding with the ADV antigen peak determined by CIEP. All fractions were ultracentrifuged at 243,000*g* for 90 min, and the pellets were examined in the electron microscope. Numerous 23-nm particles identical to those seen by Cho and Ingram were found in fractions 8 and 9 only (Fig. 2). Most particles were intact, but some seemed to be hollow as the negative contrast material penetrated the capsid and gave rise to a ring structure. All other fractions were negative for virus in the electron microscope, probably because of the lower ADV infectivity titres seen outside the main peak. It is interesting that the top fraction in all gradients was positive for ADV by CIEP. This could be because either ADV–antibody complexes were incompletely dissociated or non-infectious ADV antigen was found at a low density because of the absence of nucleic acid. No intact or hollow virions were seen in the electron microscope in the top fraction. A large amount of protein debris was, however, present in this region, and this could have masked recognisable virus particles. These results agree with those of Cho and Ingram as to density and morphology of their ADV antigen and provide quantitative infectivity data to indicate that the particles banded in CsCl gradients at a density of 1.37 are indeed those of ADV.

Others^{10,11} have reported a buoyant density value of 1.21 for ADV. It is not clear why our value differs from this. In the conditions of centrifugation used by Yoon *et al.*¹¹ (30–60% sucrose gradient), the maximum density particle that would band at equilibrium would be 1.28 g ml⁻¹, the density of 60% (w/w) sucrose. Unless the virus particles were extremely large, centrifuging for only 2.5 h at 38,000 r.p.m. would not allow equilibrium to be attained in any case. It seems likely that velocity, rather than equilibrium, centrifugation was performed, and the location of the suspected virus material in the gradient was not an indication of the true buoyant density of the virus. Density values as low as 1.21 could also be explained by the association of lipids with the virus particle. The infectivity of ADV is not, however, affected by extraction with ether. This strongly implies an absence of lipid in the infectious particle.

The size, morphology and density of ADV reported here are consistent with the suggestion that ADV is a member of the parvovirus group (DNA-containing)^{1,12}. The type of nucleic acid present in the virion has not been demonstrated. It is likely that with the availability of a simple purification technique this and other basic information on properties of ADV will be readily forthcoming.

BRUCE CHESEBRO
MARSHALL BLOOM
WILLIAM HADLOW
RICHARD RACE

National Institutes of Health,
National Institute of Allergy and Infectious Diseases,
Rocky Mountain Laboratory,
Hamilton, Montana 59840

Received January 10, 1975.

- ¹ Ingram, D. G., and Cho, H. J., *J. Rheumatol.*, **1**, 74–92 (1974).
- ² Eklund, C. M., Hadlow, W. J., Kennedy, R. C., Boyle, C. C., and Jackson, T. A., *J. Infect. Dis.*, **118**, 510–526 (1968).
- ³ Porter, D. D., and Larsen, A. E., *Proc. Soc. exp. Biol. Med.*, **126**, 680–682 (1967).
- ⁴ Porter, D. D., Larsen, A. E., and Porter, H. G., *J. exp. Med.*, **130**, 575–589 (1969).
- ⁵ Cho, H. J., and Ingram, D. G., *Infect. Immunity*, **8**, 264–271 (1973).
- ⁶ Porter, D. D., Larsen, A. E., and Porter, H. G., *Am. J. Path.*, **71**, 331–338 (1973).
- ⁷ Cho, H. J., and Ingram, D. G., *J. Immun.*, **108**, 555–557 (1972).
- ⁸ Cho, H. J., and Ingram, D. G., *Nature new Biol.*, **243**, 174–176 (1973).
- ⁹ Cho, H. J., and Ingram, D. G., *J. Immun. Methods*, **4**, 217–228 (1974).
- ¹⁰ Crawford, T. B., *Fedn Proc.*, **32**, 842 (1973).
- ¹¹ Yoon, J. W., Kenyon, A. J., and Good, R. A., *Nature new Biol.*, **245**, 205–207 (1973).
- ¹² Tinsley, T. W., and Longworth, J. F., *J. gen. Virol.*, **20**, 7–15 (1973).

C-type virus antigens detected by immunofluorescence in human bone tumour cultures

In several animal species, C-type oncogenic RNA viruses (oncornaviruses) cause malignant tumours of mesodermal origin, such as leukaemia, lymphomas and different kinds of sarcoma¹. C-type oncornaviruses have rarely been observed in human mesenchymal neoplasms using electron microscopy^{2,3}, but it has been claimed that molecular-biological methods can demonstrate in the tumours the presence of entities, the nucleic acids of which have sequences in common with the genomes of known mammalian oncornaviruses^{4–7}. There have also been claims that C-type viruses can be detected in tissue cultures of human tumours^{8–10}, although it seems that a contamination with an animal virus had occurred in these cases. Recently¹¹ an agent with type C RNA tumour virus characteristics has been isolated from cultured human acute myelogenous leukaemia cells.

A common cytoplasmic antigen has been repeatedly observed in cultures of human sarcomas with sera from sarcoma patients or their relatives^{12–15}. The cross-reactive nature of



Fig. 1 Human chondrosarcoma culture stained with a guinea pig antiserum to the major polypeptide of the SLV at dilution 1:20. For indirect immunofluorescence the microscope slides were sprayed with Teflon, while they were covered with a template, leaving eight wells open for incubation. Five of the wells were filled with different dilutions of the antiviral antiserum. One well was filled with the appropriate normal serum, and two wells with phosphate buffered saline (PBS). The slides were incubated for 30 min at 37 °C in a humid atmosphere, and washed thrice in PBS. After this, seven wells were incubated with the respective FITC-conjugate for 30 min at 37 °C; one well, previously incubated with PBS, was again incubated with the buffer. After washing and drying, the slides were embedded in Elvanol, and screened for granular cytoplasmic fluorescence with a Leitz Orthoplan microscope using Ploem illumination. A CS100 W/2 lamp was used for epi-illumination through a 40/1.0 objective (Zeiss). Narrow-band excitation and emission was performed using the following filter combination: 2 × KP 490 nm, BG 38/4, GG 455 nm, TK 495 nm (interference dividing plate) and SAL 525 nm as barrier filter. Microphotographs were taken with an Orthomat camera on Ilford HP 4 or Agfachrom 50S professional film. Tumour cell cultures originating from fresh tumour material (Dutch Bone Tumour Registry) were maintained in the Pathological Laboratory, Leiden. Control animal cell lines and cells infected with an animal oncornavirus were maintained at the Radiobiological Institute TNO, Rijswijk. To avoid contamination of the human cell lines with an animal virus, no animal cell lines were kept in the Pathological Laboratory, Leiden. For routine electron microscopy the tumour cell cultures were fixed *in situ* and removed from the bottle by scraping with a rubber spatula. No conspicuous sign of the presence of C-type virus was found in thin sections of these cells. Samples from many subcultures were cultured on microscope slides in Leighton tubes. When confluency was almost reached, the cells were fixed in cold acetone (–20 °C) and after washing in phosphate-buffered saline, stored in a deep freezer.

Table 1 Endpoint titration for immunofluorescence with antisera to mammalian C-type oncornavirus

Cell lines	Rat/RLV No. 45	Rat/RLV No. 76	Guinea-pig/SLV	Goat BoLV
BALB/c embryonic fibroblast (EF)	—	—	—	—
Rhesus monkey kidney	—	—	—	—
Bovine lung embryonic fibroblast (LEF)	NT	NT	—	—
Three human adult fibroblast lines	—	—	—	—
Human embryonic kidney	—	—	—	—
BALB/c EF+RLV	2,560	5,120	80	—
Woolly monkey fibrosarcoma	160	80	1,280	—
Bovine LEF+BoLV	NT	—	—	160
Several human bone tumour lines	20–160	—	40–80	—

Cultures were grown in minimal essential medium (Flow, Irving, Scotland) supplemented with glutamine, non-essential amino acids, antibiotics and 20% foetal bovine serum (Flow). The serum concentration was gradually diminished to 10% after a few days. The cultures were regularly tested for the presence of *Mycoplasma*¹⁹; all lines have proved to be negative so far. The isoenzyme patterns and the chromosome complements of the tumour lines have been studied. The cells proved to be human and not HeLa²⁰. Antisera to RLV were prepared by hyperimmunisation of adult WAG/Rij rats with cells from a syngeneic lymphosarcoma induced by this virus²¹. The antisera were tested by immunodiffusion and immunofluorescence for their reactivity and specificity. Only those antisera which in immunofluorescence gave a positive fluorescence in BALB/c murine embryonic fibroblasts infected with RLV at a titre of 1:2,560 or higher and no reaction at any serum dilution with uninfected cells were used for the study of human tumour cultures. The antisera were also tested for the interspecific, so-called gs-3 reactivity by immunofluorescence on cat embryonic fibroblasts infected with the feline leukaemia virus²² or on rat embryonic fibroblasts, treated with 5-bromodeoxyuridine and dimethylsulphoxide for the induction of endogenous C-type virus²³. All high titre anti-RLV sera were positive in both tests, giving a reaction up to an average endpoint of 1:160. A guinea-pig antiserum to the major polypeptide of the helper virus (SLV) in the woolly monkey sarcoma virus complex²⁴, was kindly provided by Dr R. V. Gilden (Flow, Rockville, Maryland)²⁵. Its specificity was assessed by a strong reaction with a culture from a woolly monkey fibrosarcoma and negative reactions with cultures from tissues of different primate species. An antiserum to the bovine leukosis virus (BoLV) was obtained from a goat with a lymphatic leukaemia induced by neonatal inoculation with BoLV. The antiserum was kindly provided by Professor A. Ressang, Centraal Diergeneeskundig Instituut, Rotterdam. It does not react with uninfected bovine or ovine embryonic fibroblasts, but gives a significant reaction with infected cultures. Fluorescein-isothiocyanate (FITC)-conjugated antisera to the immunoglobulins of various species (Nordic N. V., Tilburg) were first tested for specificity using immunoelectrophoresis. Only those slides in which the wells incubated with normal serum, FITC-conjugate only or phosphate-buffered saline (PBS) were negative, were subjected to further study. A positive and negative control was included in every experiment. They consisted of cells infected with the virus to which an antiserum was directed and the corresponding uninfected cell line.

this antigen has been considered indicative of a virus because different C-type viruses isolated from the same species have strong cross-reactive antigenicity. The C-type oncornaviruses isolated from different mammals usually have some antigenic determinants in common^{18–19}. No cross reactivity has, however, been reported between the common human sarcoma antigen and mammalian oncornaviruses. We report that, using immunofluorescence with human bone tumours, we regularly detected antigens which have determinants in common with the Rauscher murine and simian leukaemia viruses (RLV and SLV).

Table 2 Correspondence in positive fluorescence of human bone tumour cultures with anti-RLV and anti-SLV

Human bone tumour cell line	No. of passages	Rat/RLV No. 45	Guinea pig/SLV
Osteosarcoma	1	4	1:40
		5	1:40*
		30	—
		31	1:80*
Giant cell tumour	1	9	—
	4	8	1:40*
Chondrosarcoma	1	7	1:20*
Chondroblastoma	1	1	1:20*
Adamantinoma	1	1	—

*Some cells positive.

Using indirect immunofluorescence, cultures were screened for the presence of different cytoplasmic C-type oncornaviral antigens (see legend to Fig. 1). Table 1 shows that the various antisera do not react with uninfected animal cell lines. They give a very strong reaction with cultures infected with the viruses to which the antisera are directed, but there is a significant cross reaction between RLV and SLV. The bovine leukosis virus (BoLV) did not show a cross reaction with either RLV or SLV.

Only one out of six rat antisera to RLV tested so far reacted with several human bone tumour cultures. The negative results with the other high titre antisera excludes a possible contamination with RLV. The fact that these antisera gave a good positive gs-3 reaction is an indication that the human cultures are not infected with the feline leukaemia virus or the endogenous rat virus.

The woolly monkey virus antiserum also gave a positive reaction with several human bone tumour cultures (Fig. 1). In those cases in which the results with the rat anti-RLV serum (No. 45) could be compared with those of SLV, there is a good correspondence (Table 2). As rat anti-RLV serum (number 76) reacts with a SLV-producing culture but not with any human bone tumour culture, it can be deduced that the human cell lines are also not contaminated with SLV. Tumour lines, which reacted with anti-RLV or anti-SLV serum, did not react with the goat serum that specifically reacts with the BoLV. This suggests that the cells are not contaminated with BoLV present possibly in foetal bovine serum. As this antiserum is not very strong, more studies with high titre antisera are needed.

The fluorescence with anti-RLV or anti-SLV varied per passage as shown for two tumour lines in Table 3. Most cells were positive in some passages, in others only a few cells and, in several passages no positive cells could be detected. On continued passage, however, bright fluorescence reoccurred. Our general experience is that especially early passages are highly positive. The positive fluorescence of human bone tumour cultures with patients' sera also varied per passage^{13–15}. Positive fluorescence was observed in one or more passages in cultures of two osteosarcomas, four out of five giant cell tumours, one chondroblastoma, two out of four chondrosarcomas. One adamantinoma was negative during several passages.

To test further the specificity of the reaction with human tumour lines, the antisera were absorbed with RLV isolated

Table 3 Variation in positive immunofluorescence of human bone tumour cultures with anti-SLV per passage

Giant cell tumour 4	Osteosarcoma 1	Adult fibroblast 3
Passage	Passage	Passage
3	2	2
5	4	5
6	5	11
7	11	13
8	15	14
	20	17
	30	—
	31	—

*Some cells positive.

Table 4 Absorption tests for the specificity of the immunofluorescence reaction of human bone tumour cultures with rat anti-RLV serum No. 45

Absorption procedure	Endpoint titration	
	BALB/c EF+RLV	Human bone tumour cultures
None	2,560	80
<i>In vitro</i> with RLV*	40	—
<i>In vitro</i> with MTV*	640	40
In RLV-infected BALB/c mice†	80	—
In normal BALB/c mice†	640	20

*Antiserum was mixed with an equal volume of virus in buffer (concentration 1 mg ml⁻¹) and stored overnight at 4 °C. Thereafter, the mixture was ultracentrifuged at 100,000g for 1 h and then tested in the immunofluorescence test. An additional control was the mixing of antiserum with an equal volume of PBS and subjecting this mixture to the same procedure.

†Antiserum (0.5 ml) was injected into 8 week old BALB/c mice or into BALB/c mice with RLV-induced erythroblastosis of the same age.

from plasma of leukaemic mice²⁶ or with murine mammary tumour virus isolated from tumours²⁷. An *in vivo* absorption experiment was also performed. Both absorption procedures markedly reduced in the immunofluorescence assay on RLV-infected cells and completely blocked the reaction with human tumours (Table 4). The control absorptions only slightly diminished the reaction in both systems, indicating the specificity of the reaction of human tumour cells with antiserum No. 45. In the same way absorption of the antiserum to SLV with SLV, isolated from tissue culture fluid, blocked the reaction with human tumour cultures completely, whereas absorption with the murine mammary tumour virus had only a slight effect.

The results suggest that cultures from human bone tumours harbour a viral entity, that occasionally produces antigens which have determinants in common with known animal C-type oncornaviruses. It is not likely that the observed reactions result from contamination with murine, feline, bovine viruses or SLV. A more extensive study is needed with a greater variety of antisera to purified proteins from many different animal viruses, to assess the relationship of the putative human virus to the other viruses and to detect whether it is endogenous or exogenous. Also more sensitive techniques than the immunofluorescence method we used are needed to detect viral antigens. Furthermore, tissue culture systems must be developed which lead to a more constant production of antigen and, hopefully, to the production of detectable amounts of C-type particles. Even then, the association of this virus with the causation of bone tumours remains to be established.

We thank Miss M. Persant Snoep, Mrs J. M. Nooyen-van Ruyven and Mr A. M. Aarsen for assistance.

C. ZURCHER

Institute for Experimental Gerontology TNO

J. BRINKHOF

P. BENTVELZEN

Radiobiological Institute TNO,
Rijswijk (Z. H.)

J. C. H. DE MAN

Pathological Laboratory,
University of Leiden,
Leiden, The Netherlands

Received December 12, 1974.

- Gross, L., *Oncogenic Viruses*, second ed., (Pergamon, Oxford, 1970).
- Morton, D. L., Hall, W. T., and Malmgren, R. A., *Science*, **165**, 813 (1969).
- Stewart, S. E., et al., *J. natn. Cancer Inst.*, **48**, 273 (1972).
- Kufe, D., Hehlmann, R., and Spiegelman, S., *Science*, **175**, 182 (1972).
- Hehlmann, R., Kufe, D., and Spiegelman, S., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 435 (1972).
- Hehlmann, R., Kufe, D., and Spiegelman, S., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1727 (1972).
- Gallo, R. C., Miller, N. R., Saxinger, W. C., and Gillespie, D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3219 (1973).
- Priori, E. S., Dmochowski, L., Myers, B., and Wilbur, J. R., *Nature new Biol.*, **232**, 61 (1971).
- McAllister, R. M., et al., *Nature new Biol.*, **235**, 3 (1972).
- Stewart, S. E., Kasnic, G., Draycott, C., and Ben, T., *Science*, **175**, 198 (1972).
- Gallagher, R. E., and Gallo, R. C., *Science*, **187**, 350 (1975).
- Eilber, F. R., and Morton, D. L., *J. natn. Cancer Inst.*, **44**, 651 (1970).

- Giraldo, G., et al., *J. exp. Med.*, **133**, 454 (1971).
- Priori, E. S., Wilbur, J. R., and Dmochowski, L., *J. natn. Cancer Inst.*, **46**, 1299 (1971).
- Moore, M., Witherow, P. J., Price, C. H. J., and Clough, S. A., *Int. J. Cancer*, **12**, 428 (1973).
- Geering, G., Aoki, T., and Old, L. J., *Nature*, **226**, 265 (1970).
- Gilden, R. V., and Oroszlan, S., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1021 (1972).
- Schäfer, W., Pister, L., Hunsmann, G., and Moennig, V., *Nature new Biol.*, **245**, 75 (1973).
- Levine, E. M., *Expl Cell Res.*, **74**, 99 (1972).
- Nelson-Rees, W. A., Fliandermeyer, R. R., and Hawthorne, P. K., *Science*, **184**, 1093 (1974).
- Geering, G., Old, L. J., and Boyse, E. A., *J. exp. Med.*, **124**, 753 (1966).
- Hilgers, J., Nowinski, R. C., Geering, G., and Hardy, W., *Cancer Res.*, **32**, 98 (1972).
- Grollé, P. F., Bentvelzen, P., and Noord, M. J. van, *Biomedicine Expr.*, **19**, 148 (1973).
- Deinhardt, F., Wolfe, L., Massey, R., Hoekstra, J., and McDonald, R., *Bibl. Haematol.*, **39**, 258 (1973).
- Gilden, R. V., Oroszlan, S., Summers, M., and Davis, J., in *Possible Episodes in Eukaryotes* (edit. by Silvestri, L. G.), 98 (North-Holland, Amsterdam, 1973).
- Chenaille, Ph., Tavittian, A., and Boiron, M., *Eur. J. Cancer*, **3**, 511 (1968).
- Calafat, J., and Hageman, P. C., *J. gen. Virol.*, **14**, 103 (1972).

Mechanism of an action of an antibacterial murine lymphokine

THE inactivation and clearing of infectious agents through adaptive immune responses results from interactions between T lymphocytes, B lymphocytes and mononuclear phagocytes¹⁻⁴. The effector cells in cell-mediated immunity (CMI) are primarily T lymphocytes and mononuclear phagocytes^{1,5,6}. CMI responses appear in two stages, lymphocyte activation with subsequent production of biologically active mediators, and recruitment and activation of other cells (particularly mononuclear phagocytes) as a result of the activity of several of these mediators¹⁻³. Collaboration between T lymphocytes and macrophages in bacterial infections mediated by a lymphokine that causes inhibition of the growth of organisms within the macrophage has been assayed^{1,5,8}. This inhibition probably results from increased macrophage microbicidal activity^{1,5}. The subject of this report is a lymphokine that reduces the viability of bacteria. The mechanism of action of this lymphokine is impairment of the ability to divide of otherwise metabolically active bacteria.

The antibacterial activity was assayed on a diaminopimelic acid (DAP)-dependent auxotrophic mutant of *Escherichia coli*, cultured in DAP-free supportive medium. DAP is utilised in the synthesis of the pentapeptide normally involved in cross-linking of polymeric macromolecules in cell walls and as a source of intermediates essential for lysine synthesis⁹. Incubation in DAP-free medium resulted in formation of spheroplasts and filamentous forms and gradual termination of protein synthesis. Supernatants containing lymphokine were prepared by activation of lymphocytes with a streptococcal filtrate¹⁰ and by the lympho-

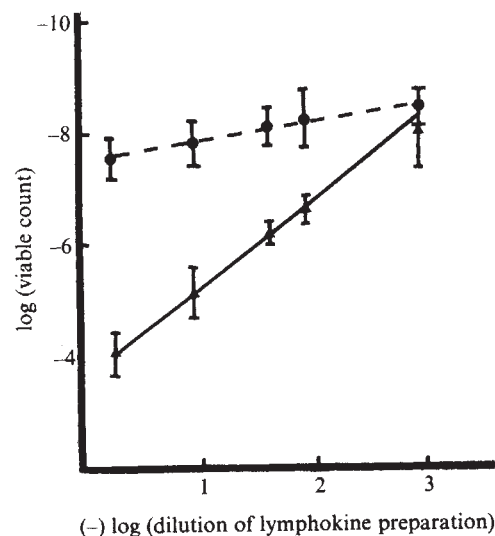


Fig. 1 Dose-dependent reduction in viable count of bacteria exposed to the purified lymphokine. The maximum ratio (control:lymphokine-treated) was approximately 2,000:1 at the highest concentration. ●, Control; ▲, lymphokine-treated.

Table 1 Incorporation of labelled metabolites into bacterial cell macromolecules and corresponding viable counts in cultures exposed to purified lymphokine and control preparations

Metabolite and isotope	Lymphokine (c.p.m.)		Control (c.p.m.)	
	(expt. 1)	(expt. 2)	(expt. 1)	(expt. 2)
Incorporation in deprived cultures (3 h pulse)				
¹⁴ C-amino acids	961 ± 17	63 ± 28	564 ± 308	1,200 ± 937
³ H-uridine	25,667 ± 3,700	1,081 ± 177	172,800 ± 91,700	94,600 ± 31,800
³ H-thymidine	3,690 ± 1,480	102 ± 30	2,180 ± 420	1,174 ± 410
Incorporation during recovery (24 h pulse)				
¹⁴ C-amino acids	39,430 ± 6,610	42,080 ± 9,460	54,900 ± 16,800	51,750 ± 1,970
³ H-uridine	200,400 ± 47,200	128,370 ± 28,960	338,700 ± 2,210	135,330 ± 32,800
³ H-thymidine	5,910 ± 1,290	62.0 ± 18.0	19,220 ± 2,210	4,060 ± 1,180
DAP-dependent viable count	7.8 ± 1.2 × 10 ⁷	5.9 ± 0.8 × 10 ⁴	5.2 ± 1.5 × 10 ⁸	1.01 ± 0.6 × 10 ⁸
NON-DAP-dependent viable count	9.1 ± 0.8 × 10 ⁵	0	6.0 ± 0.8 × 10 ⁴	1.40 ± 0.9 × 10 ²
(Colonies on TSA without DAP)				

Bacteria were suspended at a density of 10⁹ organisms ml⁻¹ in DAP-free supportive medium containing lymphokine or control preparations and incubated for 10 d. At the end of this period, 0.5 ml of culture suspensions were added to 0.5 ml of medium containing 0.1 µCi of labelled tracer (Amersham), and incubated for 3 h. At the end of the incubation, culture suspensions were diluted 1:10 in supportive medium containing DAP, and 0.5 ml of this incubated with tracer as above for 24 h to assay recovery. Viable counts were performed on DAP-containing Tryptose Soy Agar (TSA) plates. Processing and determination of incorporation of label was after the method of Bartholomaeus *et al.*¹². To optimise incorporation of ³H-thymidine, 250 µg ml⁻¹ deoxyadenosine was added with the isotope as an inhibitor of cytoplasmic thymidine kinase⁷.

cyte phytohaemagglutinin (PHA)-coated L-cell coculture method¹¹. Lymphocyte preparations (greater than 95% non-phagocytic small mononuclear cells) consisted of spleen lymphocytes prepared on Ficol-Isopaque gradients¹² mixed with equal numbers of thymus lymphocytes. Lymphokine-containing and control supernatants were concentrated on a UM 10 membrane in an Amicon Diaflo apparatus. The lymphokine was purified by Sephadex G-200 gel filtration and Pevicon block electrophoresis.

The purified lymphokine was trypsin-sensitive and gave a single band on analytical acrylamide gel electrophoresis and on immunoelectrophoresis. No cross reaction with an antiserum to foetal bovine serum could be detected. The lymphokine had a molecular weight (from Sephadex gel filtration) of 75,000 ± 15,000 and electrophoretic mobility similar to that of a fast α globulin. The lymphokine produced in lymphocyte-PHA-L-cell cocultures could not be differentiated from the lymphokine produced by stimulation of lymphocytes with streptococcal filtrates.

In cultures exposed to the lymphokine there is a reduction in viable count differing from cultures exposed to control preparations by factors up to 2,000 (Fig. 1). Exposure of wild-type *E. coli* to the lymphokine throughout a growth cycle produced an increased rate of loss of viability in stationary phase (R.L.P.F. K.J.T., and M.P.A., unpublished).

In the assay utilising the DAP-dependent mutant both lymphokine-treated and control cultures contain metabolically active organisms capable of incorporating similar amounts of labelled amino acids into protein, and uridine into RNA in recovery of metabolic activity when provided with limiting nutrient after deprivation (Table 1). The drop in viable count in lymphokine-treated cultures correlates with the almost total loss of the capacity to incorporate ³H-thymidine into DNA in cultures which remain DAP-dependent (Table 1). These results are consistent with the hypothesis that the primary effect of the lymphokine is impairment of bacterial nucleic acid metabolism expressed in the inhibition of the synthesis of new DNA, and thus, loss of the ability to divide and consequent drop in viable count. In deprived cultures the effect extends to depression of RNA metabolism. This suggests that transcription as well as DNA replication may be inhibited.

The relationship between this lymphokine and the factor that inhibits the growth of microorganisms detected in the serum of immune mice after challenge with antigen¹³ is of considerable interest. Investigation of the relation between these factors will give a clear indication of any involvement of lymphokines in direct antibacterial immune responses. Nonspecific production

of lymphokines in response to supernatants from streptococcal cultures would suggest that some nonspecific responses to bacteria¹⁴ may be mediated similarly by lymphokines.

R. L. P. FLOWER
K. J. TURNER
M. P. ALPERS

Department of Microbiology,
University of Western Australia,
Perth Medical Centre,
Shenton Park 6008, Western Australia

Received December 20, 1974; revised February 17, 1975.

- WHO Scientific Group on Cell Mediated Immunity and Resistance to Infection, WHO Monograph (September, 1972).
- Allison, A. C., *Proc. R. Soc. Med.*, **66**, 1151-1154 (1973).
- Bloom, B. R., *Adv. Immun.*, **13**, 101-208 (1971).
- Hill, H. R., Gerrard, J. M., Hogan, N. A., and Quie, P. G., *J. clin. Invest.*, **53**, 996-1002 (1974).
- Fowles, R. E., Fajardo, I. M., Leibowitch, J. L., and David, J. R., *J. exp. Med.*, **138**, 952-964 (1973).
- Blanden, R. V., *Transplant. Rev.*, **19**, 56-88 (1974).
- North, R. J., *Cell. Immun.*, **7**, 166-176 (1973).
- Krahenbuhl, J. L., Rosenberg, L. T., and Remington, J. S., *J. Immun.*, **111**, 992-995 (1973).
- Graham, R. K., thesis, Univ. Western Australia (1968).
- Seravalli, E., and Taranta, A., *Cell. Immun.*, **8**, 40-54 (1973).
- Kolb, W. P., and Granger, G. A., *Cell. Immun.*, **1**, 122-132 (1970).
- Bartholomaeus, W. N., and Keast, D., *Aust. J. exp. Biol. med. Sci.*, **50**, 603-609 (1972).
- Salvin, S. B., Nishio, J., and Shonnard, J. T., *Infect. Immunity*, **9**, 631-635 (1974).
- Cowan, S. T., *J. Path.*, **48**, 545-555 (1939).

Corrigenda

In the article "Origin of fifteen cosmic dust particles intercepted by Pioneer 8 and 9" by J. W. Rhee, O. E. Berg, F. F. Richardson and S. Auer (*Nature*, **252**, 555; 1974), the second sentence should read "The two spacecraft are in direct heliocentric orbits with perihelia of 0.99 AU and 0.75 AU and aphelia of 1.09 AU and 0.99 AU, respectively."

In the article "Re-interpretation of Devensian Till stratigraphy of eastern England" by P. A. Madgett (*Nature*, **253**, 105; 1975), there was an error in Fig. 2. The abscissa should read

$$\frac{\text{Epidote}}{\text{Epidote} + \text{Zircon}} \times 100$$

and not as printed.

Erratum

In the article by J. de Leiris, L. H. Opie and W. F. Lubbe (*Nature*, **253**, 746; 1975), the title should read "Effects of free fatty acid and glucose on enzyme release in experimental myocardial infarction".

matters arising

Distance to Cygnus X-1

CHENG *et al.*¹ have recently re-examined our estimates^{2,3} of the distance to Cygnus X-1. They have noted that the Cepheid V547 Cyg is at $d = 6.6$ kpc, yet has colour excess 1.1, far less than predicted by a uniform reddening extrapolation. On this basis they conclude 'the only independent distance check (on Cyg X-1) is so widely in error.' It is well known (see refs 4, 5) that the dust responsible for interstellar reddening is stratified in layers, and thus for every non-zero galactic latitude there exists a distance beyond which stars show no further colour excess regardless of modulus. In such fields one therefore expects the most distant observable stars to have colour excess no greater than that of intermediate distance objects; V547 Cyg is entirely consistent with this concept. It is also wrong to state that there exist no other independent estimates of distance, as we pointed out previously. Evidence based on spectroscopic modulus⁶, linear polarisation⁷, cluster membership⁸ and interstellar lines⁹ all indicate $d > 2$ kpc. Each of these methods has its own assumptions which may be individually questioned and evaluated; none of the methods, however, uses the same assumptions as the colour excess method we used. We find it quite convincing that five independent methods, although none individually of extreme accuracy, are all in agreement.

Cheng *et al.*¹ speculate that the "possibility" of X-ray heating of the primary makes distance estimates uncertain. The amount of heating is a directly measured quantity and has been shown to be entirely trivial¹⁰. They also state that an extrapolation of the reddening diagram to beyond 1 kpc is unwarranted. But if one adopts $d \leq 1$ kpc as they claim as a possibility, then no extrapolation is necessary, and one must then postulate that this star is situated behind interstellar matter which creates reddening per unit distance far exceeding all other of the ≈ 125 objects we measured within 50' of Cyg X-1. The alternative of circumstellar rather than interstellar reddening is excluded by the lack of infrared excess in HDE226868. Furthermore, the polarisation agreement⁷ means that this excess material, implied by the authors to be in a small globule, manages in a fraction of a pc to exactly compensate for the linear polarisation otherwise provided by 2 kpc of interstellar matter.

Although we agree that Cyg X-1 is not proven to be a black hole, and other models for the system are still viable, we think that the overwhelming weight of observational evidence places this object at $d > 2$ kpc regardless of its nature.

BRUCE MARGON
STUART BOWYER
ROBERT KRAFT

University of California,
Space Sciences Laboratory, Berkeley and
Lick Observatory, Santa Cruz

- ¹ Cheng, C.-C., Phillips, K. J. H., and Wilson, A. M., *Nature*, **251**, 589-590 (1974).
- ² Margon, B., Bowyer, S., and Stone, R. P. S., *Astrophys. J. Lett.*, **185**, L113-L116, (1973).
- ³ Bregman, J., *et al.*, *Astrophys. J. Lett.*, **185**, L117-L120 (1973).
- ⁴ FitzGerald, M. P., *Astr. J.*, **73**, 983-994 (1968).
- ⁵ Sandage, A., *Astrophys. J.*, **178**, 1-24 (1972).
- ⁶ Walborn, N., *Astrophys. J. Lett.*, **179**, L123-L124 (1973).
- ⁷ Hiltner, W. A., *Astrophys. J. Suppl.*, **2**, 389-462 (1956).
- ⁸ Crawford, D. L., Barnes, J. V., and Warren, W. H., *Astr. J.*, **79**, 623-625 (1974).
- ⁹ Smith, H. E., Margon, B., and Conti, P. S., *Astrophys. J. Lett.*, **179**, L125-L128 (1973).
- ¹⁰ Mauder, H., *Astr. Astrophys.*, **28**, 473-475 (1973).

Indirect methods for detecting γ -ray bursts from supernovae

PORTER¹ suggests the possibility of detecting γ quanta from supernova bursts with a detector located at sea level. He bases his calculations on the results of a Monte Carlo computation procedure for electromagnetic showers². It follows from the tables of cascade functions² that the mean particle number behind the cascade curve peak decreases with depth t as $e^{-\lambda t}$, where λ is much smaller than $\lambda_{\min}$ the minimum cross section of photon absorption in the matter. This result is at variance with the assertion that the shower development at low levels is determined by absorption of photons with the greatest permeability³. The experimental data used by Messel and Crawford² produced a discrepancy for photon absorption in air compared with the calculations of Ivanenko⁴, which they explained by the fact that the Compton scattering had been inaccurately included in his work. Since all essential processes are accurately included in the Monte Carlo procedure, the disagreement with the cascade curves obtained by the momentum method^{5,6} was ascribed² to

the low accuracy of the reconstruction of the function based on a limited number of momenta

Numerical integration of the cascade theory one-dimensional equations^{7,8} makes it possible to include accurately all processes and to calculate the longitudinal shower development down to

Table 1 1 MeV photon absorption coefficient

Element	$\lambda_{\min}$	λ_{calc}	λ_{MC}
Air	0.61	—	0.04 (ref. 2)
C	0.60	0.53 ± 0.01	—
Fe	0.41	0.33 ± 0.01	—
Cu	0.40	—	0.38 ± 0.02 (ref. 11)
			0.11 (ref. 2)
			0.24 ± 0.02 (ref. 4)
Pb	0.27	0.21 ± 0.01	0.25 ± 0.02 (ref. 12)
			0.27 ± 0.03 (ref. 13)

sufficiently low depths to produce a good estimate of the absorption coefficient λ . Inclusion of annihilation of positrons would result in an appreciable increase of the calculation volume because the two-equation system must be replaced by a three-equation system. The contribution from annihilation was, therefore, estimated. The terms, including particle redistribution resulting from annihilation, were inserted into the system of two transport equations, and the positron number was taken as 0.4 and 0.5 times the total charged-particle number in two separate runs. The calculations were carried out for an electromagnetic shower in lead where the annihilation effect is greatest. Annihilation γ quanta from stopped positrons were also included. The estimate shows that the contribution from annihilation may be neglected to an accuracy of 3-5%. This result is in line with other calculations^{9,10}.

A lower boundary for λ can be obtained when numerically integrating the one-dimensional equations, since the inclusion of scattering will only increase the cascade particle absorption with depth. For $E > 10$ MeV in heavy elements $E > 1$ MeV in light elements, the scattering effect on the cascade curve shape may be neglected^{5,6}. We have calculated the cascade curves in lead, iron, and carbon from a primary (electron or photon) with energy E_0 in the 1-10³ GeV range for several values of E from 316 MeV to 10 KeV. The calculated and

Monte Carlo data on the $E > 1$ MeV photon absorption coefficient λ (refs 4, 11–13; Table 1) are in agreement. The difference of the calculated and Monte Carlo λ from $\lambda_{\min}$ can be explained by the effects of Compton scattering and multiple Coulomb scattering.

Comparison between the cascade curves for the various threshold energies has shown that the data tabulated in ref. 2

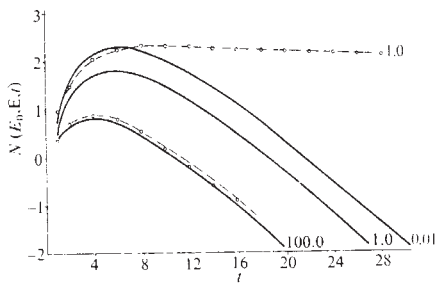


Fig. 1 The mean number of photons, $N(E_0, E, t)$ with energies exceeding E from a primary photon with $E_0 = 10$ GeV in carbon at depth t . The solid curves show the results of numerical integration, the dashed curves represent the data from ref. 2. The numerals on the curves indicate the threshold energy E in MeV.

agree with the results obtained using the numerical integration and momentum method^{5,6} for $E > 10$ MeV in lead and $E > 100$ MeV in air. Figure 1 shows the typical cascade curves from Messel and Crawford's tables for air and those calculated in the present work. The results obtained cast doubt on the conclusion drawn by Porter¹ about the possibility to detect the bursts from supervovae with a mountain or sea-level detector, since the $E > 1$ MeV photon number in ref. 2 is overestimated by a factor of 10^2 at mountain level and by a factor of about 10^4 at sea level.

A. A. BELYAEV
V. V. GUZHAVIN
I. P. IVANENKO

*Institute of Nuclear Physics,
Moscow State University,
Moscow 117234, USSR*

- ¹ Porter, N. A., *Nature*, **245**, 367 (1973).
- ² Messel, H., and Crawford, D. E., *Electron-Proton Shower Distribution* (Pergamon, Oxford, 1970).
- ³ Belenky, S. Z., and Ivanenko, I. P., *Usp. fiz. Nauk*, **69**, 591 (1959).
- ⁴ Nagel, H. H., *Z. Phys.*, **186**, 319 (1965).
- ⁵ Ivanenko, I. P., and Samosudov, B. E., *Sov. nucl. Phys.*, **5**, 622 (1967).
- ⁶ Ivanenko, I. P., and Samosudov, B. E., *Izv. Akad. Nauk SSSR, ser. fiz.*, **30**, 1951 (1966).
- ⁷ Belyaev, A. A., and Ivanenko, I. P., *Vest. Mosk. gos. Univ., ser. fiz. i astr.* (in the press).
- ⁸ Thielheim, K. O., and Zöllner, R., *J. Phys. A.*, **5**, 1054 (1972).
- ⁹ Guzhavin, V. V., Ivanenko, I. P., and Levitan, A. E., *Can. J. Phys.*, **46**, 209 (1968).
- ¹⁰ Yuda, T., et al. *Nuovo Cimento*, **65A**, 205 (1970).

- ¹¹ Völkel, U., *DESY Rep.* 65/6 Hamburg (1965).
- ¹² Woischung, W., and Burmeister, H., *Z. Phys.*, **177**, 151 (1964).
- ¹³ Borkovsky, M. Ya., and Kruglov, S. P., Preprint FTI, No. 313 (Univ. Leningrad, 1971).

Solar flare X-ray polarisation

BROWN *et al.*¹ have argued that the "Intercoms-1" and "Intercoms-4" results of solar flare X-ray polarisation measurements are in error due to incorrect 'normalisation' procedure. The normalisation was made in order to eliminate the effect of possible differences in the sensitivity of the photon counters and it is based on the assumption that the X-ray flux is unpolarised during the decay stage of flare².

According to ref. 1 the Thompson scattering of intrinsically isotropic and unpolarised flare emission taking place in the lower parts of the solar atmosphere could give polarisation P up to some 30% of observed X-ray flux (the sum of primary and scattered radiation). This conclusion is based on consideration of the scattering at 90° . In fact, because of weak dependence of the Thompson cross section on the scattering angle χ the observed flux corresponds to a wide range of χ (Fig. 1). Integration over all possible χ gives a rather low value of P (ref. 3). The area of the photosphere just under the flare gives scattered flux polarised mainly in trans-

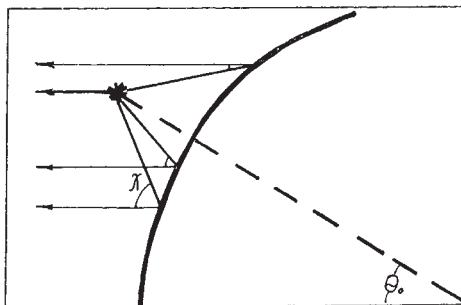


Fig. 1 Scattering of the flare X rays by the solar photosphere.

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

verse direction ($P < 0$), while the flux from more remote areas has a preferentially radial electric vector ($P > 0$). The resulting value of P depends on the altitude of the flare $\tau = H/R_\odot$ and on the heliocentric angle θ (Fig. 1).

Detailed calculations of the back scattered radiation in the first order scattering approximation are given in ref. 3. For the total flare X-ray flux we always have $|P| \leq 2\%$ (ref. 3 and see Fig. 2). Multiple scattering could alter this value by as much as a factor of two (ref. 3). It was taken into account in the computation of solar X-ray albedo using the Monte Carlo method⁴, but the

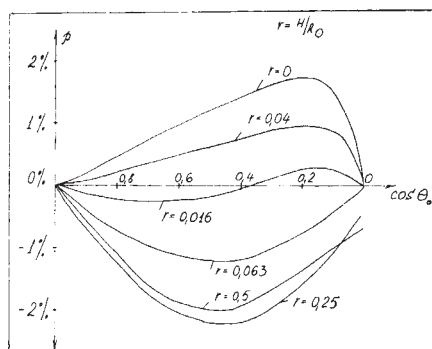


Fig. 2 Polarisation P of the total X-ray flux as function of heliocentric angle θ .

statistical significance achieved was too poor to determine the polarisation.

So inclusion of Thompson scattering effects cannot substantially influence the results of normalisation in ref. 2.

In further experiments⁵ the scatterer and the counters were mounted on a rotating drum. This permits continuous checking of the relative sensitivities and thus eliminates the need for normalisation. Unfortunately, we were not able to obtain absolute values of P with the polarimeter on board "Intercoms-7" because of the failure of one of its measuring channels. The polarimeter of "Intercoms-11" has worked well and results obtained will be published elsewhere.

S. L. MANDEL'STAM
I. L. BEIGMAN
I. P. TINDO

*P. N. Lebedev Physical Institute,
Academy of Sciences, Moscow, USSR*

- ¹ Brown, I. C., McClymont, A. N., and McLean, I. S., *Nature*, **247**, 441 (1974).
- ² Tindo, I. P., Ivanov, V. D., Mandel'stam, S. L., and Shuryghin, A. I., *Solar Phys.*, **14**, 204 (1970).
- ³ Beigman, I. L., *Astron. Zh.*, **51**, 1017 (1974).
- ⁴ Santangelo, N., Horstman, H., and Horstman-Moretti, E., *Solar Phys.*, **29**, 143 (1973).
- ⁵ Tindo, I. P., Mandel'stam, S. L., and Shuryghin, A. I., *Solar Phys.*, **32**, 469 (1973).

Are centromeric dots kinetochores?

EIBERG¹ described a Giemsa staining technique which reveals specific paired dots in the centromere region of human chromosomes in cells treated with colcemid and hypotonic KCl. He suggested that the dots may represent organelles associated with spindle fibres. Evans and Ross² demonstrated similar, stained or unstained dots obtained in colcemid-treated mammalian cells by other preparatory techniques. They hypothesised that these dots "may represent the kinetochores and particularly their associated proteins".

These interpretations are unlikely for several reasons. First, the location of the unstained dots shown by Evans and Ross² does not coincide with that of the raised dots on unstained, shadowed chromosomes. Unstained dots adjoin chromosomes in the centromere region, whereas raised dots, like Eiberg's¹ C₄ dots, lie clearly within the chromosomes. Second, there is no evidence that the centromerically located spindle microtubules are always present in colcemid-treated cells. A two hour exposure of rat kangaroo cells to 0.05 $\mu\text{g ml}^{-1}$ colcemid destroys all microtubules and alters the fine structure of the kinetochores³. Speculation that spindle proteins concentrated in the centromere regions of chromosomes could produce centromeric dots cannot, therefore, be substantiated. Third, kinetochores are rather delicate, labile organelles and they are probably affected drastically by treatments used in the preparation of chromosomes for light microscopy. For example, no kinetochores are visible in thin sections of Chinese hamster chromosomes subjected to the aceto-orcin smear technique (L. J. Journey, personal communication). Fourth, even if kinetochores were preserved by light microscopic techniques they could hardly produce prominent raised dots, for the disk-shaped kinetochores of mammalian chromosomes are only some 100 nm thick^{3,4}.

Optical and electron microscopic observations of rat kangaroo cells treated with hypotonic colcemid⁵ suggest a different interpretation of centromeric dots. Paired dots are recognisable by phase contrast microscopy in the centromere region of favourably oriented chromosomes in embedded prometaphase cells (Fig. 1a). In thin sections the dots appear as patches of chromatin packed more densely than in the remainder of the chromosomes (Fig. 1b). The kinetochores which are less opaque to electrons lie adjacent to these patches. In metaphase cells the chromosomes are, overall, more condensed, but the chromatin is also more densely packed in the centromere region than in the arms (Fig. 1c). The outer layer of each kine-

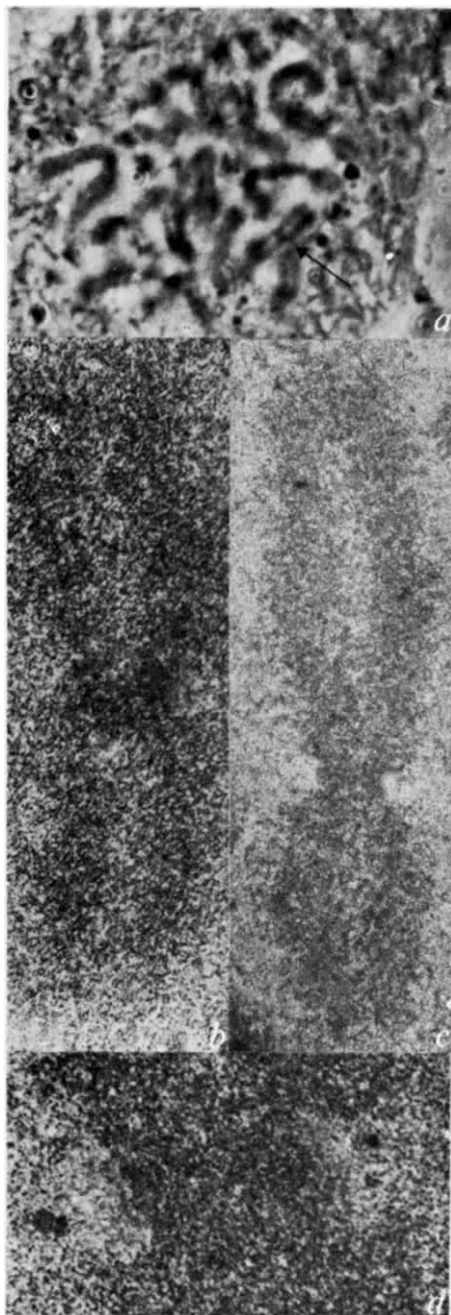


Fig. 1 Chromosomes of rat kangaroo cells (line PtK₂) treated with hypotonic colcemid (15 min at 37 °C in 0.25 $\mu\text{g ml}^{-1}$ colcemid prepared by diluting an aqueous 0.5 $\mu\text{g ml}^{-1}$ stock solution 1:1 with culture medium. See ref. 3 for culture conditions, fixation and embedding). *a*, Phase contrast micrograph of a prometaphase cell ($\times 1,625$). Note the paired dots in the centromere region of favourably oriented chromosomes. Single dots are visible on laterally viewed chromosomes. *b*, Thin section of the arrowed chromosome of Fig. 1a ($\times 11,700$). Note the two patches of densely packed chromatin in the centromere region. *c*, Metaphase chromosome $\times 7,475$. Note the dense centromeric chromatin and the adjoining electron-lucent zones. *d*, Centromere region of another metaphase chromosome $\times 22,425$. Note the kinetochore bands at the surface of the primary constriction and the electron-lucent zones.

tochore appears as a moderately electron-opaque band lying parallel to the chromosomal surface in a distinctly electron-lucent, approximately circular zone adjacent to the centromere region (Fig. 1c and d).

These electron-lucent zones correlate with, and may be equivalent to, the unstained dots described by Evans and Ross². This interpretation is supported by Journey's observations (personal communication) of "empty", circular zones adjacent to the centromere region of Chinese hamster chromosomes in thin sections of aceto-orcin smears. The densely packed centromeric chromatin, on the other hand, may account for the dots revealed by Eiberg's¹ Giemsa staining technique and by the shadowing technique of Evans and Ross². Dense structures reminiscent of centromeric dots are also seen in the centromere region in whole-mount preparations of Chinese hamster chromosomes treated with distilled water⁶ and in whole mounts of HeLa chromosomes stained with phosphotungstic acid⁷. All these results can be explained by a greater resistance of the centromeric chromatin to the spreading forces to which chromosomes are subjected in a hypotonic medium. This resistance may reflect a specific DNA-protein composition of the centromeric chromatin, that in turn could account for the specific staining, as pointed out by Eiberg¹.

U.-P. Roos

Département de Biologie Végétale
University of Geneva,
1211 Geneva 4, Switzerland

¹ Eiberg, H., *Nature*, **248**, 55 (1974).

² Evans, H. J., and Ross, A., *Nature*, **249**, 861-862 (1974).

³ Roos, U.-P., *Chromosoma*, **41**, 195-220 (1973).

⁴ Jokelainen, P. T., *J. Ultrastruct. Res.*, **19**, 19-44 (1967).

⁵ Roos, U.-P., thesis, Univ. Florida (1971).

⁶ Stubblefield, E., and Wayne, W., *Chromosoma*, **32**, 262-294 (1971).

⁷ Moses, M. J., and Counce, S. J., *J. exp. Zool.*, **189**, 115-120 (1974).

Logic of animal conflict

MAYNARD-SMITH and Price have shown¹ that it is not necessary to invoke group selection to explain the occurrence of 'conventional' rather than 'dangerous' tactics in animal conflict. The conditions for the evolution, under individual selection, of populations in which conventional tactics predominate are, however, more stringent than they suggest. In particular, given their pay-off matrix, the population may end up consisting mainly of individuals adopting the 'dangerous' strategies, Hawk and Bully.

Consider a population consisting almost entirely of Hawk and Bully, so that nearly all conflicts are between these. Then

Hawk is the better strategy when the opponent is Bully and Bully is better when the opponent is Hawk. Thus we have a system of frequency-dependent selection, leading to a stable mixture of Hawk and Bully. At this equilibrium, Hawk and Bully will have equal fitness on average and will therefore have frequencies: Hawk 0.575652; Bully 0.424348. In nearly all conflicts, the opponent will be Hawk or Bully, with frequencies just given, so that average pay-offs will be: Mouse 19.5000; Hawk 20.4311; Bully 20.4311; Retaliator 11.3932; Prober-Retaliator 13.6357.

Therefore, types other than Hawk and Bully are at a disadvantage and will not spread. These results will not be affected by the presence of a few individuals adopting strategy Mouse for non-genetic reasons, since Hawk and Bully are the best (equally good) strategies when the opponent is Mouse.

If, for simplicity, we regard the five strategies as reproducing asexually with fitnesses given by average weighted pay-offs, we find that the Hawk-Bully equilibrium is attained from some starting points, for example, with strategies given in the order above, (0.33, 0.33, 0.33, 0.005, 0.005) or (0.9, 0.025, 0.025, 0.025, 0.025). On the other hand, starting with all strategies of equal frequency, the ultimate population consist entirely of Retaliator. These results suggest strongly that some modification of the original model is required to explain the general occurrence of conventional strategies.

J. S. GALE

L. J. EAVES

Department of Genetics,
University of Birmingham,
PO Box 363,
Birmingham B15 2TT, UK

¹ Maynard-Smith, J., and Price, G. R., *Nature*, **246**, 15 (1973).

PROFESSOR MAYNARD-SMITH REPLIES—I am afraid that Gale and Eaves are quite right. They have found an alternative evolutionary stable strategy to the conflict which the late Dr Price and I investigated.

Department of Biology,
University of Sussex,
Falmer, Brighton BN1 9QG, UK

Anti-Darwinism among the molecular biologists

OHATA¹ asserted that evolutionary change at the macromolecular level was caused primarily by "mutation pressure" rather than Darwinian selection. This view, upheld by an influential school of biochemists and molecular biologists (most of them named in Ohta's bibliography), strikes some of us as regressive and potentially dangerous to our science.

One major danger is that it will dissuade biochemists from looking for functional significances in sequence differences (for example those of cytochrome *c* from different species). There is also a more general danger, that of encouraging ahistorical, statistical and mathematical thinking at the expense of the search for causal and historical explanations of the particularities of organisms.

It was a Scottish philosopher of science who wrote that "Only in the last resource, when the heavenly powers fail us, should resort be had to the demons of the underworld, chance and probability"². In the case of macromolecular evolution, it does not seem to me that Ohta and those who think like him in any way have demonstrated the failure of the "heavenly powers" of Darwinian selection. Earlier, Kimura and Ohta³ had coined the phrase "naive pan-selectionism" for the ideas of those who disagreed with them, and this phrase was taken up by Wills⁴ in a paper, not cited in Ohta's bibliography, which criticised in some detail the arguments of Kimura and Ohta, and showed that the evidence was quite consistent with a selective origin for most, if not quite all, the protein differences met with in nature.

The major argument cited by the followers of Ohta and Kimura in favour of their attitude has been the alleged time-proportionality in the numbers of residues differing between species, for cytochrome *c* and other proteins. Reasons have been put forward, such as the "Red Queen hypothesis" of van Valen¹³, why a loose proportionality of this sort might be expected on the basis of ordinary selectionist theory. Ohta, however, treats it as an established fact that there exists a "proportionality far too accurate to be explained in this way. It needs to be pointed out that, in the graphs published to illustrate this proportionality, by Dickerson⁵ for cytochrome *c*, for example, and by Wilson *et al.*⁶ for haemoglobins, the apparent linearity of the relationships depends on the assignment of some very questionable ancestral ages for the taxa concerned. Thus Dickerson gives a mid-Cretaceous age (around 100 Myr) for the ancestries of the main orders of placental mammals, whereas fossil evidence⁷ would place this in the very late Cretaceous or early Palaeocene, perhaps 75 Myr ago. For a common ancestry of mammals and reptiles, that is an ancestral amniote, Dickerson cites a Lower Carboniferous (around 320 Myr) age, whereas fossil evidence⁷ would suggest an early Permian age of perhaps 270 Myr. For a common ancestor of Amphibia and Amniota, Dickerson's graph assigns a late Devonian age of some 350 Myr, whereas fossil evidence⁷ would rather suggest the mid-Carboniferous, some 300 Myr ago. Finally, the age given by Dickerson for a common ancestor of insects and vertebrates is late Pre-

cambrian, perhaps 600 Myr old. This would be about the age of the celebrated Ediacara fauna of Australia, described by Glaessner⁸ in which quite advanced annelid and possible primitive types of arthropods are represented; undoubtedly, any common ancestry of the deuterostomian line (including Vertebrata) and the molluscan-annelid one from which the insects sprang must have been very considerably older, perhaps more than 700 Myr old.

The similar graph for the haemoglobins given by Wilson *et al.*⁶ assigns some even more questionable ancestral dates—for example, original separation of the cyclostome (lamprey) line from that of Gnathostomata, is placed in the Proterozoic, some 800 Myr ago, probably nearly as old as the entire metazoan line!

A further assumption of Ohta is that protein polymorphism in natural populations is non-adaptive and a result of mutation pressure. Where such polymorphism has been studied in detail for particular proteins, as pointed out by Johnson¹², the phenomena have been found to parallel closely those of polymorphism in ordinary phenotypic characters, selective control of which has been demonstrated, as pointed out by Ford¹¹ in another important and relevant work not mentioned in Ohta's bibliography. Selander and Kaufman⁹, also not cited, draw attention to a book by Levins¹⁰ in which the theory is developed of the long term adaptive advantages of maintaining certain critical degrees (varying with the characters and the circumstances) of heterozygosity of natural populations.

Ohta's evident pride in the mathematical sophistication of his methods of analysis prompts a scriptural gloss on his phrase "naive pan-selectionism": namely, that by adopting what he would consider as the naivety of "little children", the molecular biologists might improve their chances of entering into that "kingdom of heaven" in which the historic truths of evolution are revealed.

R. A. CROWSON

Zoology Department,
University of Glasgow,
Glasgow G12 8QQ, UK

¹ Ohta, T., *Nature*, **252**, 351–354 (1974).

² Ritchie, A. D., *Studies in the History and Methods of Science* (Edinburgh University Press, 1958).

³ Kimura, M., and Ohta, T., *Nature*, **229**, 467–469 (1971).

⁴ Wills, C., *Am. Nat.*, **107**, 23–34 (1973).

⁵ Dickerson, R. E., *Scient. Am.*, **226**, 58–75 (1972).

⁶ Wilson, E. O., and Eisner, T., *Life on Earth* (Sinauer Associates Inc., Stamford, 1973).

⁷ Harland, W. B., *et al.*, *The Fossil Record*. Geological Society, London, 1967.

⁸ Glaessner, M. F., *Rec. S. Aust. Mus.*, **13**, 369–401 (1959).

⁹ Selander, R. K., and Kaufman, D. W., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1875–77 (1973).

¹⁰ Levins, R., *Evolution in Changing Environments* (Princeton University Press, New Jersey, 1968).

¹¹ Ford, E. B., *Ecological Genetics*, third ed. (Chapman and Hall, London, 1971).

¹² Johnson, G. B., *Nature*, **242**, 193 (1973).

¹³ van Valen, L., *J. molec. Evol.*, **3**, 89–101 (1973).

reviews

As a result of intensive research since the beginning of the present century, the traditional image of the Middle Ages as an epoch of sterile subservience to the authority of the Church and of Aristotle in scientific matters has been destroyed. Instead, it is now recognised as a period during which scholars became capable of wide ranging and subtle, albeit habitually inconclusive, speculation upon topics commonly supposed to be the distinctive concern of early modern science—the rotation of the Earth, for example, the atomic structure of matter, the possibility of creating a vacuum, and so on. It has emerged, moreover, that such mediaeval speculations, although cast in a very different mould, are by no means isolated from the articulation of the new philosophy in the 17th century. On the contrary, as Edward Grant clearly illustrates in *A Source Book in Medieval Science*, scholastic discussions exercised a patent or possible influence on such later innovators as Copernicus, Galileo and Otto von Guericke. At the other chronological extreme, Professor Grant begins his anthology of mediaeval science with extracts from the late-classical encyclopaedias that preserved an inkling of ancient culture in the Latin west during the Dark Ages, and proceeds thence to illustrate in considerable detail subsequent developments in logic, mathematics, physics, cosmology, chemistry, geography, biology and medicine. Although the bulk of the material is drawn from the period 1200–1500 AD to have documented approximately a millenium of, at times, intense intellectual activity

Medieval record

C. J. T. Lewis

A Source Book in Medieval Science. Edited by Edward Grant. Pp. xviii+864. (Harvard University Press: Cambridge, Massachusetts, August 1974.) \$32.50.

over such a wide range of subjects is obviously no mean achievement.

It is not only the sheer bulk of available material that renders the construction of an anthology of mediaeval science so problematic a task, however. In addition, there is the difficulty of selecting topics and texts that are relevant both to the subsequent evolution of early modern science and to the interests of the ordinary 'educated reader' at whom this work is partly directed, without simultaneously distorting the emphasis of the original speculations. Professor Grant is, of course, well aware of, and for the most part effectively reconciles, such conflicting demands.

Nevertheless, it is perhaps inevitable that he should tend towards the use of an essentially positivist criterion in making his selections. This tendency is most conspicuous in the devotion of relatively little space to alchemy and astrology, and the complete exclusion of the more exotic magical sciences. More subtly, however, the same criterion determines a concentration on the 'achievements' of mediaeval science

that I have already mentioned. Occasionally, this leads to a measure of distortion, most obviously, for example, in the documentation of mediaeval 'kinematics'.

On the whole, however, such misrepresentation is avoided; the broader context of individual problems, the characteristic conceptual interrelationships and the typical methods of mediaeval science are illustrated with considerable force and economy. Indeed, a particular virtue of this source book is that the (to a modern mind) metaphysical preoccupation, and the unique gothic complexity, of much scholastic scientific discussion is clearly illustrated by the reproduction of complete *quaestiones* and treatises.

It is inevitable in a work of such size and scope that one should take exception to certain features and register certain omissions. Thus, although the copious annotation of individual documents renders them perfectly comprehensible, it is regrettable that the sections on physics should be provided with neither a contemporary nor a modern outline of the principles of Aristotelian natural philosophy, which constituted, after all, the foundation of most scholastic physical speculation. Again, it seems a pity, considering the possible audience of this book, that further references for given topics are not more thoroughly recorded. Ultimately, however, it must be said that this book offers a clear and scholarly exposition of a mass of fascinating documents, and provides an unparalleled insight into the processes and pre-occupations of mediaeval science. □

MOST students find mathematical physics a difficult subject. They can follow a qualitative explanation (such as, "magnetisation lines up the dipoles"), and they can follow the steps in an algebraic deduction, or in the solution of an equation and the evaluation of an integral; the physics and the mathematics are philosophically distinct activities. Difficulties arise, however, at the meeting-point between these disciplines. How does one mathematise a physical problem? Why do the same equations crop up over and over again, in apparently unrelated physical contexts?

This original undergraduate text is a largely successful attempt to answer the second of these questions. The philosophy is to apply the field equa-

tions of mathematical physics (Laplace, Poisson, wave, and diffusion) to problems of increasing complexity, illustrating the solutions obtained with examples from a very wide range of

Maths and physics

Michael Berry

Introductory Eigenphysics: An Approach to the Theory of Fields. By C. A. Croxton. Pp. x+275. (Wiley: London and New York, November 1974.) £6.95 board; £3.95 paper.

fields of physics. First, the field equations are introduced. Then they are solved in rectangular, cylindrical and spherical coordinates. Finally, approximate methods (perturbation, WKB and variational) are discussed. The origin-

ality lies in the examples chosen which are oriented towards physics rather than applied mathematics and which include lattice vibrations, Bloch waves, skin effect, aerofoil theory, vibration of a rotating star, earthquakes in the Mohorovicic layer, pseudopotentials in liquid metals, and quantum chemistry. There are many interesting exercises for the reader.

Unfortunately, the book is seriously marred by many minor errors and infelicities, most of which could have been avoided by careful proof-reading. Symbols are not defined, figures are imperfectly labelled and terms are introduced without explanation. This will confuse all but the best students. I do, however, warmly recommend the book to teachers. □

Agriculture and industrialisation

Agriculture and the Industrial Revolution. By E. L. Jones. Pp. xiii+233. (Blackwell: Oxford, February 1975.) £5.50.

As the title suggests, this is a book about the relationships between the development of agriculture in Britain and the process of industrialisation which we refer to as the Industrial Revolution. The work, a collection of essays published by the author during the 1960s, is divided into two main sections. A group of essays concerned mainly with agricultural change and the process of industrialisation constitutes the first part, and a further group round the theme of agriculture in the urban industrial economy comprises the second. Many of the essays are detailed studies of agricultural changes in specific locations, as, for example, are the pieces on farming in Hampshire and Herefordshire. But the author is able to bring a considerable degree of theoretical clarification to his materials. This is nowhere more clearly exemplified than in the essay entitled "Agriculture and Economic Growth in England 1650-1815: Economic Change". In this essay the author takes up vigorously the problem of explaining the essential relationships between agriculture and the Industrial Revolution. In particular he has tried to separate, for the purpose of analysis, the "longer term causes of agricultural change in the centuries before the Industrial Revolution, and agriculture's role in the Industrial Revolution through such activities as capital formation, the release of productive factors and demand expansion". The author concludes that, on balance, the agricultural sector made a valuable net contribution to economic growth during the period 1650-1815. He confirms that the typical textbook argument, for example, that labour was ejected into factory industry by the force of the parliamentary movement, needs closer scrutiny.

There is no space in a review as short as this to venture into the rich detail of the essays nor is there space even to give a brief summary of each one. It may, perhaps, serve to draw attention to the contemporary importance of this volume, if I select a passage from R. M. Hartwell's introduction and indicate the range of questions that the author treats. Did the agricultural revolution produce an industrial revolution or *vice versa*? Did more people produce more food or more food, more people? Did an agricultural surplus allow the growth of industry or did industrial change induce agricultural change? What was the relationship of

the agricultural revolution to the Industrial Revolution and to the growth of population?

The use of the past tense in the forming of questions should not, however, obscure their contemporary relevance. Three quarters of the world is trying to achieve modernisation through some combination of agricultural and industrial inputs. Further, the balance chosen as a matter of policy is not infrequently frustrated by a seemingly uncontrollable population expansion. Though no one expects that in the modernisation of the Third World history will (or must) repeat itself, intellectuals and the more practically oriented alike should peruse the pages of this volume if they seek to understand the fine structure of the contemporary problem they are attempting to solve.

Michael Gibbons

Human problems

Ethical, Social and Legal Dimensions of Screening for Human Genetic Disease. Edited by Daniel Bergsma. Pp. viii+272. (Symposia Specialists: Miami, 1974. Distributed by Stratton International Medical Book Co., New York.) \$13.95.

THE rise of interest in genetic screening over the last few years has probably been very largely the result of technological developments. As Tabitha Powledge says in her contribution to this work by members of the Genetics Group of the Hastings Institute of Society, Ethics and Life Sciences, "It is regrettably true that, just because we can do something, we very often proceed to do it, without thinking much about whether we *should*". A recurring theme throughout this book is that we should have a very clear idea of why we want to screen, quite apart from whether or not it is practical. According to Powledge, and her sentiments are echoed by other contributors, research is a rational and legitimate purpose of screening but in that case screening should not be presented as having a service or of being of direct benefit to the screened.

There are, perhaps, two important reasons for screening for genetic disease. First, if there is an effective treatment which, instituted early enough, could prevent the development of severe mental handicap, for example, in phenylketonuria. Second, so that genetic counselling and, where possible, advice on antenatal diagnosis can be given early enough to prevent the possible birth of another affected child in the family. In initiating such screening programmes it is, however, important to keep in mind the general principles of Wilson and Jungner (*Principles and Practice of*

Screening for Disease; World Health Organisation: Geneva, 1968), in particular the provisos that the disorder must be an important health problem, it must be treatable (and/or preventable), the screening test must be reliable, and it must be cost-effective to screen.

In the light of these sentiments the value, and problem, of screening for unifactorial, multifactorial and chromosomal disorders are discussed. In the case of unifactorial disorders the main problem is that most are rare, and even for those that are relatively common a reliable screening test is often not yet available; for example, in fibrocystic disease. Two relatively common disorders which have been considered recently for screening are certain protease inhibitor genotypes and familial hyperbetalipoproteinaemia.

In so-called multifactorial disorders (for example, essential hypertension and diabetes mellitus) the main problem is that even if one had a reliable test for detecting preclinical cases, there is as yet little evidence to suggest that treatment at this stage would prevent the development of the disease. According to Mellman the screening of newborn populations for chromosomal abnormalities is now of doubtful justification, for apart from providing incidence figures it tells us nothing of the natural history of such disorders. The process of identifying individuals in newborn surveys and studying them from birth until social maturity is too slow to satisfy society's impatience for information on say the XYY and XXX phenotypes. Such screening would be more informative if it included school children and even samples of the adult population.

Quite apart from the scientific merit and cost-effective benefit of screening, there are the social, psychological, ethical and legal problems associated with genetic screening. Predictably, participants who volunteer for screening programmes (as in the case of Tay Sachs disease) are younger and, on average, better educated than those who do not wish to participate in such programmes. And certainly the knowledge that one is a carrier of a genetic disease may have profound effects on marriage plans and marital relationships. The legal and ethical problems of whether or not information obtained from screening programmes should be communicated to the person screened, and possibly also to his relatives if medically or genetically indicated, require the most careful consideration.

Issues raised by genetic screening are becoming increasingly important to us all. This collection of essays goes a considerable way towards making us stop to consider the implications.

A. E. H. Emery

Reflections on surfaces

Science and Technology of Surface Coating. Edited by B. N. Chapman and J. C. Anderson. Pp. xvii+463. (Academic: London and New York, 1974.) £14.80; \$41.50.

Characterisation of Solid Surfaces. Edited by P. F. Kane and G. R. Larra-bee. Pp. xviii+670. (Plenum: London and New York, 1974.) \$39.00.

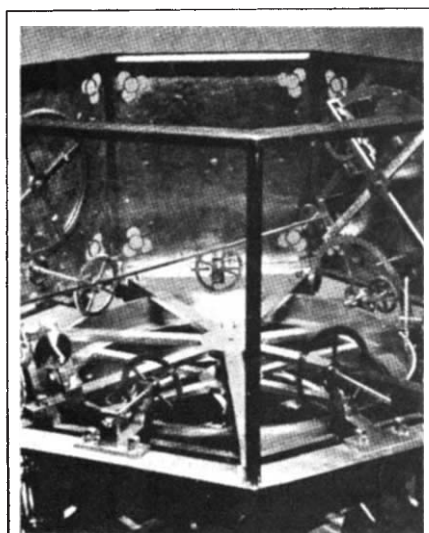
THESE two volumes bring out two immediate observations. First, that both the technology of producing surface coatings and the methods of microstructural analysis have undergone extremely rapid development. Second, that many of the new surface coating techniques still owe more to acute experimental observations and technical imagination than to direct application of scientific knowledge.

These thoughts are provoked particularly by the first volume on surface coatings, the editors of which deserve congratulations on producing in less than 500 pages 44 articles written by enthusiasts. These articles outline the bewilderingly large range of methods currently available for applying surface coatings to useful materials, and indicate some of their applications. It was clearly not possible or desirable in articles of this length to discuss any of the topics at all fully, but all of the articles give an outline of the physical principles involved and in most cases a useful list of references is included.

In addition to those topics that are predictably present—for example, electroplating, evaporation, chemical vapour deposition, and so on—there are other, more unexpected but worthwhile methods discussed. These include well established technologies such as brush painting, printing and spraying; the physics of such technologies being subjects less often considered in the scientific literature. Among the newer technologies, those of plasma arc and flame spraying and detonation bonding of refractory materials are described together with fascinating new ideas, such as that of 'electrical harmonic spraying' in which an electrical potential applied to liquid drops emerging from a tube can produce a collimated beam of liquid drops that are uniformly charged and have constant mass and velocity, such that they can be deflected accurately to form a deposited pattern without the need of a mask. Another idea that seems to be technically 'sweet' was the method of 'ion plating' in which adhesion between a substrate and a coating material can be improved by an increased kinetic energy of the first deposited layer. This increased energy is achieved by ionisation

of the evaporating atoms followed by electrical acceleration onto the substrate.

Two omissions, are, however, striking—'metalliding', the electrodeposition of metals with molten fluoride electrolyte was presented by abstract only; and no discussion was given of the Xerox process. The lack of a full discussion on metalliding was probably no fault of the editors, but at least a list of references might have been obtained from the missing author. The absence of Xerography is disappointing in that many of the coating methods discussed use charged material deposited onto a uniformly charged substrate, so selective deposition onto a partially charged



Modern reconstruction of Giovanni de Dondi's astronomical clock. The original was made in about 1364. From *Gears from the Greeks. The Antikythera Mechanism—A Calendar Computer From ca. 80 BC.* By Derek DeSolla Price. Pp. 70. (Neale Watson Academic: New York, 1975.) \$8.50.

insulator seems a natural extension of these ideas.

The major criticism, however, must be that only a few articles discussed the *properties* of the coatings, and only one considered their structural assessment—either the title should have been 'Methods of Producing Surface Coatings', or more consideration of the properties of coatings should have been given.

Characterisation of Solid Surfaces is a very different volume in which a smaller range of topics (23) is considered in greater depth. The analytical methods described range from genuine atomic surface techniques such as Auger spectroscopy, electron spectroscopy for chemical analysis, and field ion microscopy, to techniques normally considered only for assessment of bulk microstructure such as transmission electron microscopy (TEM) and electron probe microanalysis (EPMA). The relevance of such bulk techniques is,

however, clearly justified in that the 'bulk' material studied—100 nm for TEM and 1 μ m for EPMA—though large compared with a single atomic depth may be appropriate to many surface applications, such as surface coatings.

In addition, as shown by Laird in his excellent discussion of TEM, much genuine surface information can be gained by suitable specimen preparation facilities. The converse of this idea is also discussed—the analysis of bulk microstructure by use of surface analysis combined with erosion techniques so that the bulk can be revealed as a series of exposed surfaces. As a result of this interchange between bulk and surface analysis the content of this book will be of value to all interested in the microstructure of materials; not just to the rapidly growing band of surface scientists.

The techniques described all involve the illumination of surfaces with photons of various frequencies, electrons, ions or neutral atoms, followed by an examination of emitted photons, electrons, ions or atoms. The varied combinations of these techniques has given rise to a large industry both supplying and using increasingly expensive and varied equipment. It is noticeable that only in the chapter on optical microscopy was the cost of the equipment considered in respect to the value of the information obtained.

The rapidly growing arsenal of expensive equipment described indicates the importance of deciding what information is really needed in any application and which method is best for obtaining it. It often seems that scientists may become oriented to using just one technique—that in which they have detailed knowledge—ignoring others that may be more useful. The level of expertise obtainable from most of the chapters in this book should do much to indicate the advantage of other techniques, though for a detailed understanding one would have to make use of the literature cited.

Among the noticeable omissions was a discussion of low energy electron diffraction, though the editors refer to a good review. Perhaps more serious in view of the seemingly inevitable delays between writing and publication was the lack of a list of important references that might have covered the interval between writing and final printing of the volume.

A final and inevitable reflection on these two volumes is the lack of application of the analytical techniques to the evaluation of the structure of surface coatings; one can only hope that these two books will lead to some cross fertilisation between these two groups of surface scientists and technologists.

R. D. Doherty

announcements

Awards

The Royal Geographical Society has announced the following awards: **Founders Medal** to **Sir Lawrence Kirwan** (geographical history of Nubian Nile Valley and Eastern Africa; services to exploration); **Patron's Medal** to **J. P. Kuettnner** (exploration of Earth's atmosphere and oceans); **Victoria Medal** to **C. O. Sauer** (geographical research and education); **Busk Medal** to **N. N. Ambraseys** (historical seismicity and recent earthquakes in Near and Middle East); **Murchison Award** to **A. L. Mabogunje** (geography of West Africa); **Back Award** to **H. C. Brookfield** (geographical studies of Pacific region); **Peek Award** to **A. S. Goudie** (Late Pleistocene climatic phases in north-west India); **Gill Memorial** to **T. W. Freeman** (geography of Ireland); **Ness Award** to **A. Thompson** (botanical surveys in Guyana); **Cherry Kearton Medal and Award** to **Eric Ashby** (nature cinematography).

Appointment

T. G. C. Murrell has been appointed Foundation Professor of Community Medicine at the University of Adelaide.

Miscellaneous

Interstellar clouds. Professor A. Dalgarno, Professor of Astronomy at Harvard College Observatory, will give two Special University Lectures on Molecular Processes in Interstellar Clouds at University College, London (Gower Street, WC1) at 5 pm on April 23 and 25.

Geodynamics Project. The British National Committee for Geodynamics wishes to produce a comprehensive listing of papers relevant to this project, published by UK workers between January 1, 1971 and the present. Would such workers please send a bibliography of their papers to the Royal Society by April 30 (6, Carlton House Terrace, London SW1Y 5AG, UK).

Standard Telecommunications Laboratory. SRC and NERC are prepared to receive applications for the use of this facility at Harlow in Essex by university research groups for high pressure geophysical and geochemical experiments (Dr V. Essex, State House, High Holborn, London WC2, UK).

Person to Person

Oxford-Stanford. Oxford scientist requires three to four bedroom house in Stanford, California for sabbatical period, August 1, 1975–May 1, 1976. Possible exchange with modern four bedroom house in north Oxford, convenient for laboratories and all university buildings (Dr W. G. Richards, Physical Chemistry Laboratory, South Parks Road, Oxford, UK).

London flat. Canadian couple urgently require accommodation (flat rental) in London area from June, 1975–December, 1976. In return for assistance offered, willing to help with accommodation problems in Montreal, Toronto or Vancouver (Dr I. Altosaar, Department of Biochemistry, Imperial College, London SW7, UK).

London-Washington. Three bedrooms, central heating, available for exchange with accommodation in Washington, DC (within reasonable distance of National Cancer Institute). Approximate dates: August 1, 1975–July 1, 1976. Contact: M. Norman, 97 Highland Road, Bromley BR1 4AA, UK.

Boston-London. Two to three bedroom accommodation required for all or part of August in London or along the south coast, by a responsible academic family (two school children) from Boston area. Exchange arranged if interested. (Christine Armett-Kibel, 18 Devon Terrace, Newton Centre, Massachusetts 02159.)

Reports and publications

Great Britain

Bulletin of the British Museum (Natural History). Entomology. Vol. 31, No. 6: A Review of the Subfamily Oxyinae (Orthoptera: Acridoidea). By D. Hollis. Pp. 189–234. £2.75. Entomology, Supplement No. 24: A Revision of the Subfamily Coelidiinae (Homoptera: Cicadellidae). Tribes Tinobregmini, Sandersellini and Tharrini. By M. W. Nielson. Pp. 1–197. £12.20. Zoology. Vol. 27, No. 5: Catalogue of the Types of Terrestrial Isopods (Oniscoidea) in the Collections of the British Museum (Natural History). 1. Superfamily Pseudotracheata. By R. J. Lincoln and J. P. Ellis. Pp. 189–246. £3.10. Vol. 26, No. 6: *Campylaspis* Species (Crustacea: Cymacea) from the Deep Atlantic. By N. S. Jones. Pp. 147–300. £3.25. (London: British Museum (Natural History), 1974 and 1975.) [271]

The Management of Broadleaved Woodlands. (Report of the Fourteenth Discussion Meeting, Institute of Foresters of Great Britain, Reading, 5 to 7 April, 1974.) (Supplement to *Forestry*.) Pp. 119. (London: Oxford University Press, 1974.) £2. [271]

Other countries

Slow Sand Filtration. By L. Huisman and W. E. Wood. Pp. 122. (Geneva: WHO; London: HMSO, 1974.) Sw.fr. 16. [211]

Agriculture Canada: Canadian Grain Commission. Grain Research Laboratory—1973 Report. Pp. vi + 92. (Ottawa: Information Canada, 1974.) [211]

Environment Canada: Fisheries and Marine Service. Technical Report No. 490: Eggs, Larvae and Juveniles of Fishes from Plankton Collections in the Gulf of St. Lawrence During 1968. By A. C. Kohler, D. J. Faber and N. J. McFarlane. Pp. 105. (St. Andrews, NB: Research and Development Directorate, Biological Station, 1974.) [211]

Académie Royale de Belgique. Mémoires de la Classe des Sciences. 2e Série, T. XLI, Fascicule 5: Acritarches de la "Tranchée de Senzeille" (Frasnien Supérieur et Famennien Inférieur). Par F. Stockmans et Y. Willière. Pp. 79 + 4 planches. (Bruxelles: Académie Royale de Belgique, 1974.) [221]

Smithsonian Contributions to Zoology. No. 175: On the Caobangiidae, a New Family of the Polychaeta, with a Redescription of *Caobangia billeti* Giard. By Meredith L. Jones. Pp. iii + 55 (11 plates). \$1.15. No. 182: Spider Crabs (Crustacea: Brachyura: Majidae) from the International Indian Ocean Expedition, 1963–1964. By D. J. G. Griffin. Pp. iv + 35. 85 cents. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) [221]

United States Department of the Interior: Geological Survey. Professional Paper 832: Geology of the Skagway B-3 and B-4 Quadrangles, Southeastern Alaska. By E. M. MacKevett, Jr., E. C. Robertson, and G. R. Winkler. Pp. iv + 33. (Washington, DC: Government Printing Office, 1974.) \$2.05. [221]

Annual Report of the Institute of Earth and Planetary Physics for 1974. Pp. 117. (Edmonton, Alberta: Institute of Earth and Planetary Physics, University of Alberta, 1974.) [231]

United States Department of the Interior: Geological Survey. Professional Paper 816: Geology of the Betterton Quadrangle, Kent County, Maryland, and a Discussion of the Regional Stratigraphy. By James P. Minard. Pp. iii + 27 + plate 1. (Washington, DC: US Government Printing Office, 1974.) \$1.20. [231]

A New Model for Level Areas. By Doeko Goosen. (Inaugural Address.) Pp. 32. (Enschede, The Netherlands: International Institute for Aerial Survey and Earth Science, 1974.) [271]

Australian Journal of Zoology. Supplementary Series. No. 31: The Generic Relationships of the Scincid Lizard Genus *Lepidopisma* and its Relatives. By Allen E. Greer, Jr. Pp. 67. No. 30: A Monograph of Australian Fleas (Siphonaptera). By G. M. Dunnet and D. K. Mardon. Pp. 273. No. 32: The Tipulidae (Diptera) of Australia. XII. The Genus *Dolichopeza* Curtis. By N. V. Dobrowolsky. Pp. 27. (East Melbourne, Victoria: Editor-in-Chief, Editorial and Publications Service, CSIRO, 1974.) [271]

Bulletin of the Fisheries Research Board of Canada, No. 189: Treatment of Fish Processing Plant Wastewater. By F. G. Claggett and J. Wong. Pp. 18. (Ottawa: Information Canada, 1974.) \$2.40. [271]

Smithsonian Contributions to Zoology. No. 166: A Checklist of the North and Middle American Crayfishes (Decapoda: Astacidae and Cambaridae). By Horton H. Hobbs, Jr. Pp. iii + 161. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$2.50. [271]

Records of the Dominion Museum, Wellington, New Zealand. Vol. 8, No. 12: New Species of Haustoriidae, Phoxocephalidae, and Oedicotidae (Crustacea: Amphipoda) from Northern and Southern New Zealand. By R. D. Cooper and A. A. Finchem. Pp. 159–179. Vol. 8, No. 13: A New Subgenus of *Rhytidia* Albers, 1860 (Mollusca: Pulmonata: Paryphantidae) from New Zealand. By F. M. Climo. Pp. 181–183. Vol. 8, No. 14: The Marine Algae of Stewart Island—a List of Species. By N. M. Adams, E. Conway and R. E. Norris. Pp. 185–245. Vol. 8, No. 15: New Species of Brittle Stars from New Zealand (Echinodermata: Ophiuroidea). By Alan N. Baker. Pp. 247–266. Vol. 8, No. 16: Risso's Dolphin in New Zealand Waters and the Identity of "Pelorus Jack". By Alan N. Baker. Pp. 267–276. Dominion Museum Records in Ethnology. No. 12: Cook Voyage 'Trading Stations' in Early Protohistoric New Zealand. By D. Wayne Orchiston. Pp. 133–156. Report of the Board of Trustees, National Art Gallery, National Museum, and National War Memorial, for the year ended 31 March 1974. Pp. 27. (Wellington, NZ: Dominion Museum, 1974.) [271]

Radiological Factors Affecting Decision-Making in a Nuclear Attack. (NCRP Report No. 42.) Pp. viii + 66. (Washington, DC: National Council on Radiation Protection and Measurements, 7910 Woodmont Avenue, 1974.) [271]